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EUROPEAN MEDICAL JOURNAL 2.3
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Welcome

A warm welcome to the summer 2017 edition of the *European Medical Journal*, which delivers a selection of high-quality research papers from a variety of therapeutic areas. Encompassing topics from inherited bone marrow failure syndromes to ovarian cancer relapse, there is something for everyone to enjoy, medical specialists and industry professionals alike.

The two features of *EMJ 2.3* will be of particular interest to the oncologists and haematologists amongst you. The first feature provides a detailed summary of the recent advances in myelofibrosis treatment, with Lupu commenting on the clinical heterogeneity of myelofibrosis and the current unmet need of these patients. Our other feature is an exploration of how to correctly diagnose hypereosinophilic syndromes.

Continuing the theme of conditions with poor prognoses, Pesántez et al. discuss the novel immunotherapeutic agents for acute lymphoblastic leukaemia, as the focus on immunotherapy in the clinic rises, whilst Giornelli and Mandó propose a theory on the role of cancer stem cells in the recurrence and relapse of ovarian cancer. Also from the field of haematology, Korkmaz details the common features of non-classic myeloproliferative neoplasms and stresses the need for disease-modifying drugs against initial diagnostic markers in an attempt to prevent transformation to acute leukaemia. Additionally, a comprehensive summary of common inherited bone marrow failure syndromes is included by Foglesong et al.

> “Encompassing topics from inherited bone marrow failure syndromes to ovarian cancer relapse, there is something for everyone to enjoy, medical specialists and industry professionals alike.”

This edition also sees a strong focus on urology: Albaz and Üçer describe the current state-of-the-art therapies for Peyronie’s disease, with the hope of an optimum therapeutic option being made available, and Aggarwal et al. explain the role of serotonin in premature ejaculation treatment. In addition, Sokhal et al. deliver a fascinating evaluation on the use of alpha blockers to facilitate negotiating the ureteroscope through the ureteric orifice during medical expulsive therapy of ureteric calculi. Moving on to interventional techniques from the field of cardiology, this edition presents papers summarising the indications, procedures, and outcomes of two effective cardiology procedures: fetal pericardiocentesis and chronic total occlusion-percutaneous coronary intervention. Finally, Mandal comments on the significance of asymptomatic bacteriuria and their potential treatment with antibiotics.

As I am sure you will agree, this recent issue of the *European Medical Journal* comprises a wide variety of research papers with content which will benefit the whole medical community. We would like to thank the authors for their contributions and the Editorial Board for their continued support, and we look forward to delivering the final edition of this eJournal to all readers later this year.

Spencer Gore

Director, European Medical Journal

Foreword

Dr Emanuele Angelucci

Chairman, Hematology and Transplant Center, Ospedale Policlinico San Martino, Istituto di Ricovero e Cura a Carattere Scientifico, Genova, Italy.

Dear Colleagues,

It is my pleasure and honour to introduce this prestigious volume of the *European Medical Journal*, which contains very important articles in various fields of medicine. All the articles are a clear representation of how 'fast and furious', and also exciting, the progress of medicine has become in recent years. I think it is a great privilege to practice medicine and undertake clinical research in these years of impressive advances in the care of our patients.

In this issue of *EMJ*, several topics in various medical disciplines are covered. Important updates with immediate practical impact are presented, including a new review of inherited bone marrow failure syndromes, an expert management opinion on primary myelofibrosis, and a new definition of disease categories for non-classical myeloproliferative neoplasm. After years of research, immunotherapy has finally entered the therapeutic protocols of acute lymphoblastic leukaemia therapy; in this *EMJ* issue you can find a complete report of immunotherapy in lymphoblastic leukaemia. As far as oncology is concerned, new issues and theoretical mechanisms of ovarian cancer relapse are presented.

In non-neoplastic diseases, recent treatment modalities of Peyronie's disease, the use of preoperative alpha-blockers, the role of selective reuptake inhibitors in premature ejaculation, intervention in chronic total occlusion-percutaneous coronary intervention, and fetal pericardiocentesis are presented and discussed.

I have chosen Gameiro et al.'s article discussing renal involvement in multiple myeloma as my Editor's Pick for this issue, because I believe it is of particular importance. This article describes advances in understanding the pathogenic mechanism of renal impairment in patients with multiple myeloma and the consequent possibility of early intervention. This article is also important because it highlights the multidisciplinary nature of haematology. Haematologic diseases involve and affect almost all tissues and organs of the human body; this, on the one hand, requires haematologists' expertise in internal medicine and, on the other hand, requires consultants with proven experience in haematology. In haematology, dedicated consultants are fundamental, as well as laboratories dedicated to the haematology wards.

Of course, all articles have been peer reviewed to ensure the quality and independence of content.

Enjoy reading!

Emanuele Angelucci

Chairman, Hematology and Transplant Center, Ospedale Policlinico San Martino, Istituto di Ricovero e Cura a Carattere Scientifico, Genova, Italy.

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Myelofibrosis (MF), either primary or secondary to polycythaemia vera or essential thrombocythaemia, is the most symptomatic and has the worst prognosis among the *BCR-ABL1*-negative myeloproliferative neoplasms (MPN). Most recent estimates of median survival range from 6–7 years, comparable to those of other haematologic malignancies, such as some chronic lymphoproliferative malignancies.[1] Patients with MF fall along a spectrum that ranges from those who are fully asymptomatic to those who have severe symptoms that affect patients' quality of life. Another characteristic of MF is that the disease is associated with progressive constitutional symptoms, increasing splenomegaly and worsening cytopenias, and increasing risk of leukaemic transformation.

MF mostly affects elderly people, the median age at diagnosis being >60 years, but there are rare cases of asymptomatic young people with a diagnosis of MF; in these cases, allotransplantation of haematopoietic stem cells is the only therapeutic method with curative intent. The clinical landscape is very diverse, with spleen enlargement, due to extramedullary haematopoiesis, and portal hypertension being responsible for abdominal discomfort, early satiety, and abdominal pain. Anaemia and constitutional symptoms can accompany MF, with fatigue the most often seen symptom. Some patients may report other symptoms. To have a more objective evaluation of the symptoms, several symptom scores have been developed. In daily clinical practice, most used is the Myeloproliferative Neoplasm-Symptom Assessment Form (MPN-SAF), which assesses 10 symptoms: fatigue, inactivity, night sweats, itching, fever, weight loss, bone pain, early satiety, problems with concentration, and abdominal discomfort.[2] This is a very useful tool to evaluate the burden of the disease dynamics. Quality of life in patients with MF is severely compromised by several constitutional symptoms (i.e. fatigue, night sweats, fever, weight loss), pruritus, and symptoms from frequently massive hepatosplenomegaly.

For MF diagnosis, we are using World Health Organization (WHO) criteria in clinical practice, which incorporates all the progress made in recent years regarding the mutational status, but also includes histological bone marrow (BM) patterns which differentiate fibrotic primary MF (PMF) from early/prefibrotic PMF.[3] The interpretation of BM histopathology features, as required by the current WHO classification, requires experienced pathologists.[1] This update of WHO criteria will probably be expected, in the future, to improve distinction of the different MPN subtypes by the histological BM patterns and corresponding clinical features.

Our understanding of MF has continuously evolved in the last decade. Numerous Janus Kinase 2 (*JAK2*) mutations and deletions are implicated in MPN; dysregulated JAK-STAT signalling plays an important role in MF disease pathology. Discovery of the Val617Phe mutation of the *JAK2* gene in 2005[4] in the majority of MF patients represented an important step towards elucidating the pathogenesis of this disease. Mutations in the thrombopoietin receptor gene (*MPL*) were subsequently found in a smaller percentage, followed, in recent years, by the discovery of mutations in the calreticulin gene (*CALR*) which have been observed in a proportion similar to Val617Phe mutation.[5] A smaller part of patients have 'triple negative' disease, which has been associated with inferior outcomes.[5] In recent years, other mutations (*ASXL1*, *SRSF2*, *IDH1/2*) associated with lower overall survival or leukaemia-free survival were described. The study of new mutations in recent

years, together with the already known prognostic scores, measured through the International Prognostic Scoring System (IPSS) used at diagnosis (which stratifies the patients into four risk groups: low and intermediate-1 groups have a longer survival compared to intermediate-2 or high-risk groups), and the Dynamic International Prognostic Scoring System (DIPSS) used in dynamics over the course of the disease, help us to have an idea about the future evolution of MF.

HOW I TREAT MYELOFIBROSIS

The main goals of therapy for PMF are the prolongation of survival and, if possible, also cure, which is currently achieved only by allogeneic stem cell transplantation (allo-SCT), but, due to its increased risks, morbidity, and mortality, it is usually restricted to eligible high and intermediate-2-risk MF patients. If prolongation of survival or cure is not possible, symptom-orientated palliation and quality of life become the primary goals of therapy. Besides the paramount progress in the biology of the disease, many efforts have been directed toward the development of molecularly targeted therapies, including inhibitors of JAK1 and JAK2. The international guidelines for MF, including those from the National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO), were updated and reflect the changes in the therapeutic area; however, the incorporation of new WHO diagnosis criteria are still required.

For low-risk and asymptomatic patients, the 'watch-and-wait' approach is still used, with careful monitoring of the patient to ensure initiation of the therapy as soon as the patient status changes. For intermediate and high-risk patients with symptomatic splenomegaly and MF-related symptoms, hydroxyurea was the most used cytoreductive drug in the past; surgery and radiation therapy were used for splenomegaly, but are associated with significant morbidity and mortality. For patients with MF and anaemia, specific drugs are used to improve haemoglobin levels: erythropoiesis-stimulating agents, androgens, or immunomodulatory agents, but the results are usually temporary and sometimes incur severe side effects, and limited availability can restrict their use. Blood transfusion can be an option to raise haemoglobin levels for some cases.

Among JAK inhibitors, several molecules are still in different phases of development. The differences in their toxicity profile and efficacy that have been reported in clinical trials suggest variability of these molecules regarding potency and target selectivity.

The only approved JAK inhibitor for splenomegaly or symptoms of MF is ruxolitinib, approved by European Medicines Agency (EMA) in 2012,[6] irrespective of the risk group or by presence of *JAK* Val617Phe mutation. Ruxolitinib is indicated for MF in adult patients who have splenomegaly or symptoms related to the disease, such as fever, night sweats, bone pain, and weight loss. It has been shown to be highly effective in ameliorating disease symptoms and reducing spleen size. In the COMFORT I pivotal study, as well as in the COMFORT II study, the primary endpoint of obtaining >35% reduction in spleen volume by imaging techniques at 24 or 48 weeks of treatment was achieved, with ruxolitinib improving overall survival versus placebo or best available therapy.[7,8] The starting dose of ruxolitinib depends on platelet count, but the dose should be titrated up as soon as possible to get the best spleen response to treatment. Even though anaemia may initially worsen during ruxolitinib treatment, the haemoglobin levels tend to improve over time, but, in rare cases, anaemia represents the reason for ruxolitinib discontinuation.[7,8] In daily practice, for some MF patients, the improvement of symptoms can minimise the impact of anaemia. The most common haematological side effects are anaemia and thrombocytopenia, which are manageable. The response to treatment with ruxolitinib in MF is illustrated by the results of the COMFORT studies, showing an improvement in overall survival, and it is therefore the best available therapy for the moment.

Despite the recent developments in understanding the molecular biology of MF and treatment with JAK inhibitors, which have shown significant clinical benefits for the patients, there remains an unmet need for the condition, due to its molecular and clinical heterogeneity with high degrees of variability from patient to patient; more research is needed to standardise or to apply a personalised approach to every patient based on molecular biomarkers.

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HOW I DIAGNOSE HYPEREOSINOPHILIC SYNDROMES

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ABSTRACT

Hypereosinophilic syndromes are a group of disorders characterised by significant eosinophilia and organ damage. They have proven challenging to define, diagnose, and study for many years, due in part to their variable clinical presentations, the overlap between neoplastic and reactive eosinophilia, and the lack of a universal marker of eosinophil clonality. Herein, we give an overview of the term and discuss aetiology and our approach to diagnosis.

Keywords: Hypereosinophilic syndromes (HES), diagnosis, eosinophils, eosinophilia.

EOSINOPHILS

Eosinophils are mature granulocytes derived from the bone marrow myeloid progenitor cells under the influence of a number of cytokines, particularly interleukin (IL)-5, though they may also mature in other organs. They are morphologically identifiable by the presence of orange granules when stained with the acidic dye, eosin. They represent approximately 1–5% of peripheral blood leukocytes (reference range: 0.05–0.5x10^9/L) and account for a similar fraction of nucleated cells within the bone marrow. The majority of eosinophils reside in tissue, primarily the thymus, lymph nodes, spleen, gastrointestinal tract, and uterus. Reference ranges for eosinophil numbers in these organs are less well-described.

Eosinophil granules contain many active compounds. Some (e.g. major basic protein, eosinophil cationic protein) exert direct cytotoxic effects. Others, including cytokines and chemokines, recruit and activate other cells including lymphocytes, mast cells, and fibroblasts.[1] When appropriately targeted, granule release helps control pathogens, while inappropriate release has been implicated in the development of allergic inflammation, fibrosis, vasculitis, and thrombosis.

HYPEREOSINOPHILIC SYNDROMES

Terminology around these disorders has undergone many changes and is frequently confusing. The term 'hypereosinophilic syndrome' was first proposed in 1968 to describe disorders with persistent eosinophilia and tissue damage due to eosinophil infiltration.[2] The definition was later refined to describe peripheral blood eosinophilia of unknown origin, exceeding 1.5x10^9/L, and persisting for at least 6 months, that was responsible for organ dysfunction/damage.[3]

A 2011 consensus panel proposal[4] distinguishes between hypereosinophilia (HE) and hypereosinophilic syndromes (HES). Under this proposal, the term 'hypereosinophilia' should be used when there is peripheral blood eosinophilia of >1.5x10^9/L, on two occasions, at least 1 month apart, or in the presence of significant tissue eosinophilia. Identification of this latter finding is recognised as being challenging given the lack of well-established reference ranges. Finally, it acknowledges that, although blood eosinophilia usually accompanies tissue eosinophilia, tissue findings may occur in isolation.

The same panel proposes the term 'hypereosinophilic syndrome' be reserved for cases that fulfil the definition of HE and have otherwise

unexplained organ dysfunction/damage. This damage may be due to direct cytotoxic effects of eosinophil granule contents, or occur secondary to recruitment of other cell types. The time requirement for diagnosis may be waived in cases of evolving and life-threatening organ damage in order to facilitate appropriate therapy.

HES may be classified into several broad groups. Primary (neoplastic) and secondary (reactive) HES are discussed below. HE of uncertain significance exists when neither primary nor secondary causes are apparent despite extensive investigation. Familial HE has also been described[5] and appears to be due to abnormal dysregulation of cytokine signalling.[6,7]

Primary (Neoplastic) Hypereosinophilic Syndromes

Primary (neoplastic) HES are defined by the presence of neoplastic (clonal) eosinophils. In the 2016 classification system, the World Health Organization (WHO) recognises two key groups of eosinophilic disorders. The first, myeloid/lymphoid neoplasms with eosinophilia and rearrangements of *PDGFRA*, *PDGFRB,* or *FGFR1*, now includes the provisional entity with *PCM1-JAK2* rearrangement.[8] In order to diagnose one of these entities, the specific genetic rearrangement must be demonstrated. The second diagnostic category, chronic eosinophilic leukaemia-not otherwise specified (CEL-NOS), is categorised within the myeloproliferative neoplasms.

Clonal eosinophils may be identified in other haematological malignancies. Core binding factor acute myeloid leukaemia (AML), particularly cases with *CBFB-MYH11* fusion, are associated with atypical eosinophil proliferation. The diagnostic genetic rearrangement has been demonstrated in eosinophils in both *CBFB-MYH11* AML[9] and in *RUNX1-RUNX1T1* AML.[10] In these cases, the diagnosis of AML takes precedence.

Secondary (Reactive) Hypereosinophilic Syndromes

Secondary (reactive) HES, in contrast, generally result from increased eosinophil production and may occur in a variety of disease conditions. The eosinophils are not clonally related and the eosinophilia is generally considered to be driven by cytokine/growth factor production. Infection is the most common cause of persistent eosinophilia worldwide.[11] Parasite infection is particularly prevalent in developing countries and in certain areas of developed countries. Eosinophilia in these conditions tends to be most marked when parasites or their products encounter immune effector cells in tissue; purely intraluminal parasitisation is less associated with persistent eosinophilia.[12]

Allergic/atopic/autoimmune

Persistent eosinophilia is commonly found in allergic and atopic disease. Its development is driven by activated Type 2 T helper cells that produce eosinophil-stimulating cytokines.[13] Autoimmune disorders, such as eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome) and granulomatosis with polyangiitis (Wegener's granulomatosis), are often associated with eosinophil infiltration of tissue. Hypersensitivity reactions, including allergic bronchopulmonary aspergillosis and the spectrum of drug reactions, may also result in HES.

Malignancy

Secondary HE associated with malignancy is most commonly connected with T cell malignancies, Hodgkin lymphoma, and B cell acute lymphoblastic leukaemia. Eosinophilia in these conditions is typically due to excessive cytokine (IL-5) production.[4]

Lymphocyte-variant hypereosinophilic syndrome

This variant of HES results from the abnormal proliferation of T helper cells. These cells are typically phenotypically aberrant, most commonly $CD3^-/CD4^+$,[14] frequently express markers of activation, and produce large amounts of IL-5, demonstrated by testing supernatant after *in vitro* lymphocyte culture.[15] Consensus diagnostic criteria for this condition are not yet established.[16] The exact incidence of this subtype has been difficult to estimate, being a rare entity, though seems likely to account for 20–25% of otherwise unexplained cases of HES.[15]

Other

A number of other causes of significant eosinophilia are described. Episodic angioedema with eosinophilia (Gleich syndrome) is now recognised to be a cell cycling disorder of uncertain aetiology,[17] with symptoms occurring at approximately monthly intervals. Eosinophilia is also seen in patients with immunoglobulin G4-related disease. Initially thought to be related to atopy, eosinophilia is reported in non-atopic patients with this condition.[18]

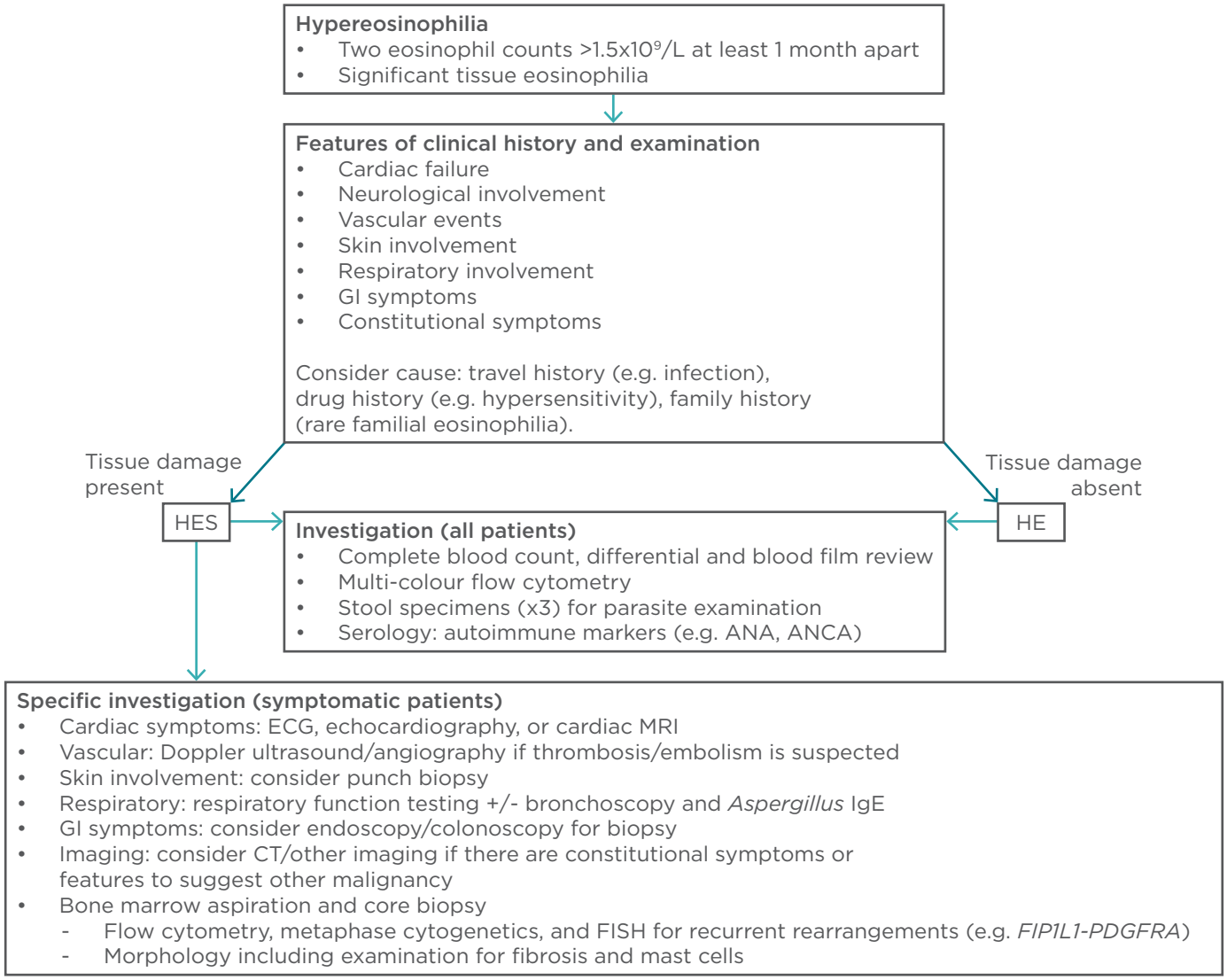

Figure 1: An overview of our approach to diagnosing HES.
ANA: anti-nuclear antibody; ANCA: anti-neutrophil cytoplasmic antibody; CT: computed tomography; ECG: electrocardiogram; FISH: fluorescence *in situ* hybridisation; GI: gastrointestinal; HE: hypereosinophilia; HES: hypereosinophilic syndromes; IgE: immunoglobulin E; MRI: magnetic resonance imaging.

It is now considered likely to be related to the disease process itself.

APPROACH

HES are defined by the presence of organ dysfunction. Our first aim in assessing a patient with HE is to identify organ dysfunction as this allows appropriate classification and facilitates treatment. Our second aim is to identify the underlying cause. The clinical history and examination findings play a key role in guiding specific investigations. An overview to our approach is provided in Figure 1.

We specifically enquire about features of cardiac failure given the occurrence of endomyocardial fibrosis in HES. Neurological involvement may be present and can affect the central nervous system (e.g. diffuse encephalopathy) or result in peripheral neuropathy. Vascular complications should be sought. HES may trigger thrombosis through endovascular damage or through direct activation of the clotting system, while embolic events from cardiac thrombi can present with acute arterial occlusion.

As some patients may overlook minor skin changes, particularly if long-standing, specific inquiry and examination are recommended. The nature of skin symptoms is highly variable, with some having cutaneous erythema or eczema while others may have more severe symptoms such as urticaria, angioedema, or even ulceration.

Respiratory symptoms may be prominent but are frequently non-specific. Cough and wheeze, associated with bronchospasm, may be seen while others may develop slowly progressive dyspnoea, associated with pulmonary fibrosis. Occasionally respiratory symptoms, with or without fever, may be clearly triggered by allergen exposure, and suggest hypersensitivity reactions.

A travel history, particularly to areas where parasite infection is endemic, and gastrointestinal symptoms (including abdominal pain, diarrhoea, and nausea) raise the possibility of HE secondary to parasitisation. Gut symptoms, including malabsorption, may also be seen as a direct consequence of eosinophil infiltration, cell damage, and fibrosis.

The presence of constitutional symptoms (night sweats, fever, >10% unintended weight loss) should prompt consideration of underlying malignancy. A detailed medication history is essential given the potential for hypersensitivity reactions to cause eosinophilia.

DIAGNOSTIC TESTING

We use an eosinophil count >1.5x10^9/L on at least two occasions 1 month apart to define HE.[4] In the absence of organ damage we take a stepwise approach to diagnosis, attempting to exclude secondary causes before investigating neoplastic processes. If organ damage is identified, we typically investigate primary and secondary causes of HES simultaneously to hasten diagnosis, allow specific therapy, and minimise further tissue damage. If organ damage is acute, severe, or progressive we often commence corticosteroid therapy concurrent with diagnostic testing.[19]

Investigation for organ dysfunction should be guided by clinical history. In asymptomatic patients with incidental HE we typically defer such investigation. In symptomatic patients, we perform investigations targeting the involved organ system(s). These may include echocardiography, computed tomography (e.g. brain, chest, abdomen), and Doppler ultrasound to exclude vascular abnormalities. Contrast-enhanced cardiac magnetic resonance imaging has been increasingly used in the evaluation of cardiac fibrosis. It is superior to echocardiography and other imaging techniques[20] and has the advantage of allowing detailed mapping of fibrosis as well as being more sensitive for the detection of ventricular thrombi. Disadvantages include the need for gadolinium contrast administration, limiting use in those with renal failure, and, in some regions, limited availability.

Tissue biopsy may be required in some cases where symptoms are partially explained by other medical conditions or where eosinophil infiltration of the affected organ is unproven. Given the challenges inherent to determining significance of eosinophil infiltration, particularly in assigning causality of organ dysfunction, discussion with the reviewing pathologist is highly recommended. Tissue biopsy may also demonstrate atypical lymphoid infiltration in cases of lymphocyte-variant HES.[21]

Blood counts and morphological review of the blood film are essential to diagnosis. Blood film review may identify abnormalities, including dysplastic eosinophils, to suggest primary HES. Secondary HES may also be morphologically identified with conditions such as acute lymphoblastic leukaemia and, rarely, circulating parasites are identifiable. Determination of serum tryptase is suggested given that its elevation is a minor criterion for the diagnosis of systemic mastocytosis.[22] Multi-colour flow cytometry should be performed in all patients. This allows detection of aberrant T cell immunophenotypes associated with lymphocyte-variant HES. Our preference is to perform this test on bone marrow though we consider peripheral blood flow cytometry in asymptomatic patients with HE.

T cell receptor (TCR) gene rearrangement studies have been increasingly used in the setting of eosinophilia to investigate T cell clonality and lymphocyte-variant HES. Clonal rearrangements are common, with one study finding 18 out of 42 (42.8%) patients to have TCR clonality.[23] Complicating matters, however, was the finding of clonal rearrangements in two patients with the *FIP1L1-PDGFRA* fusion product. This is in keeping with other work where clonal *TCR* rearrangement may be found in benign lymphoid proliferation.[24] As such, the finding of a *TCR* rearrangement, in the absence of immunophenotypic or aberrant cytokine production, should not be considered as diagnostic.[25] We recommend TCR gene rearrangement studies to confirm clonality if the diagnosis of lymphocyte-variant HES is strongly suspected; we do not recommend these studies be performed routinely in those without flow cytometric abnormalities (e.g. aberrant immunophenotype, expansion of specific lymphocyte subsets).

Microbiological investigations should be performed. Three or more fresh stool specimens for parasite examination should be obtained. Serological testing for specific parasites may be appropriate in selected cases; testing for *Aspergillus*-specific immunoglobulin E may be useful if allergic bronchopulmonary aspergillosis is suspected. In the presence of respiratory symptoms, we request respiratory function testing. In selected cases, particularly where a cause for eosinophilia remains unclear, we refer for bronchoscopic investigation to obtain diagnostic lavage and exclude pulmonary infection.

In patients with organ damage, those with lymphocytosis, immunophenotypic abnormalities by flow cytometry or morphological abnormalities (including circulating precursors and dysplasia) on blood film examination, or after exclusion of secondary causes, we perform bone marrow aspiration and trephine biopsy. The aspirate specimen should be submitted for morphological evaluation, metaphase cytogenetics, fluorescence *in situ* hybridisation, and flow cytometry. The core biopsy should be submitted for pathological review, including assessment for reticulin and collagen fibrosis. Other immunohistochemical studies may be warranted depending on findings. Specific consideration should be given to the presence of systemic mastocytosis and occult neoplasia.

High-throughput DNA sequencing does not yet have a definitive role in the diagnosis, prognostication, or identification of therapeutic targets in HES. A number of recent publications[26-28] have examined the role of sequencing in these disorders, particularly in identifying clonal molecular abnormalities in idiopathic HE, though this work has yet to influence routine clinical practice. Accordingly, we do not routinely request extended DNA sequencing in our investigative approach.

Myeloid/lymphoid neoplasms with eosinophilia and recurrent genetic rearrangements should be specifically excluded. The majority of these cases have a cryptic deletion of chromosome 4q12 creating the *FIP1L1-PDGFRA* fusion gene.[29] This cryptic deletion can be demonstrated by fluorescence *in situ* hybridisation studies. While *PDGFRB* and *FGF1* are recognised to have multiple gene fusion partners, the majority of these are detectable with metaphase cytogenetics.[30] The diagnosis of CEL-NOS may only be established after recurrent genetic lesions, including those associated with AML, have been excluded. CEL-NOS is otherwise defined by the presence of eosinophil clonality (shown by cytogenetic or molecular testing) or the presence of increased numbers of blast cells (>2% in blood; >5% in marrow).[29]

FUTURE DEVELOPMENTS

A number of important questions are, as yet, unanswered. Given the relative rarity of these diseases and previous challenges in establishing aetiology, our understanding of optimal diagnostic criteria remains based on expert opinion. International collaboration and further study may refine categorisation. Advances in diagnostic techniques may allow for direct demonstration of eosinophil clonality/polyclonality as a means of differentiating neoplastic HES from secondary/reactive eosinophilia as well as allowing disease monitoring. While eosinophils play a role in the pathogenesis of organ dysfunction, the mechanisms by which this damage occurs are poorly understood. Further research may clarify the extent of eosinophil-mediated cytotoxicity compared to the consequences of cell recruitment. Understanding these contributions may facilitate therapy to block and reverse organ damage.

SUMMARY

HES were first defined on the basis of peripheral blood eosinophilia and tissue damage. Developments in laboratory techniques have allowed for precise aetiological classification of neoplastic eosinophilia; other techniques have highlighted the role of lymphocytes and cytokine signalling in driving eosinophilia. Ongoing developments in DNA sequencing and immunophenotyping have the potential to further classify disease and identify therapeutic targets.

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MANAGEMENT OF MULTIPLY RELAPSED AGGRESSIVE NON-HODGKIN LYMPHOMA: NEW PERSPECTIVES

This symposium took place on 13th June 2017, as a part of the 14th International Conference on Malignant Lymphoma (ICML) in Lugano, Switzerland

Chairpersons

Eric Van Den Neste,[1] Ruth Pettengell[2]

Speakers

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MEETING SUMMARY

Patients with refractory/relapsed (R/R) non-Hodgkin lymphoma (NHL) make up a very heterogeneous population with a poor life expectancy. The objective of this symposium was to provide an overview of the current treatment landscape for aggressive NHL, as well as the future research on new treatments. Transplant-eligible patients receive salvage chemotherapy, followed by high-dose chemotherapy and autologous stem cell transplantation (ASCT). Patients who fail transplant or are transplant-ineligible generally receive palliative treatment or enter clinical trials; there is no standard of care and thus there is a high unmet clinical need. Pixantrone is currently indicated for adult patients with multiply R/R aggressive B-cell NHL, thereby filling the unmet clinical need in this field. The symposium started with a brief overview of the meeting objectives. This was followed by an overview of the current and future treatment landscape for aggressive NHL, including a case study of a patient with diffuse large B-cell lymphoma (DLBCL) with multiple relapses receiving pixantrone as monotherapy. The results and post hoc analysis of the CORAL and the SCHOLAR1 studies were reviewed, including the relative merits of combination therapy versus monotherapy for patients with relapsed DLBCL who had failed second-line salvage therapy. The symposium ended with an outline of the profile and mechanism of action of pixantrone, and evidence from the PIX301 study that provided the basis for regulatory approval for the use of pixantrone in third and fourth-line treatment of R/R aggressive B-cell NHL. The clinical efficacy and safety of pixantrone were reviewed, together with a future perspective on the ongoing PIX306 trial. The symposium concluded with the presentation of two clinical cases of patients treated with pixantrone, a 'Question and Answer' session, and a panel discussion.

Treatment Landscape of B-Cell Non-Hodgkin Lymphoma Patients, Looking into the Future of New Strategies

Professor Pier Luigi Zinzani

Treatment Landscape of Aggressive B-Cell Non-Hodgkin Lymphoma Patients

The majority (about 85%) of NHL arise from B-lymphocytes, with DLBCL being the most common subtype (37%).[1] Although aggressive B-cell NHL has a cure rate of approximately 60%, relapse within the first 2 years following initial therapy of rituximab with cyclophosphamide, doxorubicin (hydroxydaunomycin), vincristine, and prednisolone (R-CHOP) is common.[2]

There is currently no recognised standard of care for patients who fail first and second-line treatment and who are not eligible for SCT. Market research conducted in 2016 among a group of 154 haemato-oncologists and oncologists in France, Italy, and Spain demonstrated that ≥9 different regimens (including monotherapy and combination therapy) may be used in third and fourth-line settings.[3] The life expectancy of the multiple relapsed population is poor;[2] as such, there is a significant unmet medical need in patients with multiply R/R-DLBCL as well as those ineligible for SCT.

Prognostic Factors and New Treatment Options in Diffuse Large B-Cell Lymphoma

Gene-expression profile studies have revealed three molecular subtypes of DLBCL according to the cell-of-origin: germinal-centre B-cell-like DLBCL, activated B-cell-like (ABC) DLBCL, and primary mediastinal B-cell lymphoma.[4,5]

A number of novel single-agent therapies for relapsed DLBCL ineligible for SCT are currently in development. These include:

- Bruton's tyrosine kinase (BTK) inhibitor (ibrutinib)
- Phosphoinositide 3-kinase (PI3K) inhibitors (including buparlisib [BKM120], copanlisib [BAY80-6946], and TGR-1202)
- BCL2 inhibitor (venetoclax)[6]
- Antibody drug conjugates (denintuzumab)
- Small molecule inhibitor of exportin 1 (XPO1) inhibitor (selinexor)[7]
- Checkpoint inhibitors (pembrolizumab and nivolumab)[8]
- EZH2 inhibitor (tazemetostat)[9]
- Chimeric antigen receptor T-cell (CAR-T) therapy[10]

Overall, none of the drugs currently being evaluated show potential as an active single agent in the treatment of patients with relapsed DLBCL ineligible for SCT. However, preliminary data with CAR-T are promising; complete response (CR) rates of 50–60% have been observed in R/R-DLBCL,[11] although a number of safety issues have been noted, including cytokine release syndrome and B-cell aplasia.[10]

With regard to combination therapies, a Phase Ib/II programme evaluating combination therapies of ibrutinib, lenalidomide, and rituximab in patients with R/R non-germinal-centre B-cell-like subtype DLBCL is currently ongoing.[12]

Currently, pixantrone is the only single-agent treatment approved by the European Medicines Agency (EMA) for the management of aggressive B-cell NHL progressing after two or more prior lines of therapy.[13] The benefit of pixantrone treatment has not been established in patients when used as fifth-line or greater chemotherapy in patients who are refractory to last therapy. Pixantrone was approved by the EMA primarily on the basis of the results from the PIX301 study, which evaluated the efficacy and safety of pixantrone as a single-agent therapy in the management of patients with aggressive R/R-NHL who had received at least two prior therapies.[13,14] In a post hoc analysis of data from the PIX301 study, pixantrone was shown to be more effective than active comparator in the above-mentioned setting, independent of previous rituximab therapy.[15] In a historical comparison, pixantrone as a single agent in patients with two or more prior chemotherapy lines had a similar CR rate (about 27%) with salvage regimens, such as rituximab plus etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (hydroxydaunorubicin) (R-EPOCH) or rituximab plus ifosfamide, carboplatin, and etoposide (R-ICE), although it should be noted that the studies are not directly comparable.[14,16-19]

Case Study

In January 2010, a 36-year-old male presented with bulky axillary lymphadenopathy and was diagnosed with DLBCL with an ABC-like phenotype.[20] Computerised tomography and positron emission tomography scans demonstrated multiple lymphadenopathies above and below the diaphragm and skeletal involvement confirming Stage IV disease. The treatment plan is depicted in Figure 1.

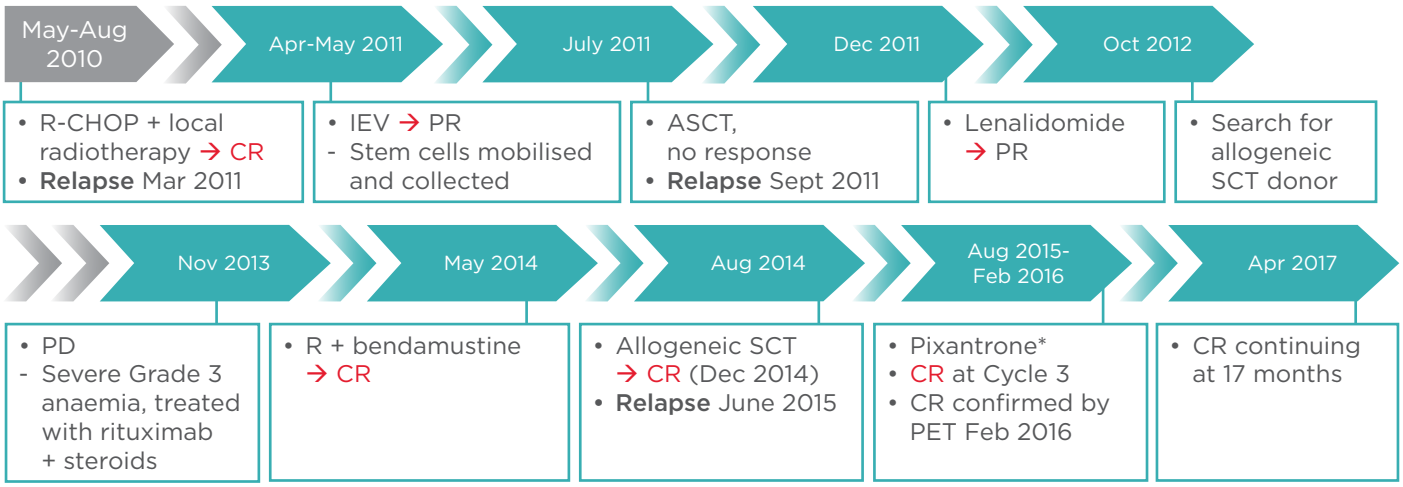


Figure 1: Case study treatment regimen.[20]

*50 mg/m^2 on Days 1, 8, and 15, for six 28-day cycles; 20% dose reduction due to risk of haematological toxicity.

ASCT: autologous stem cell transplantation; CR: complete response; IEV: ifosfamide plus etoposide and epirubicin: PET: positron emission tomography; PD: progressive disease; PR: partial response; R: rituximab; R-CHOP: rituximab plus cyclophosphamide, doxorubicin (hydroxydaunomycin), vincristine, and prednisolone; SCT: stem cell transplantation.

Following two relapses, pixantrone was administered for six 28-day cycles; CR was observed at Cycle 3. At the end of treatment, CR was confirmed by positron emission tomography and is ongoing after 17 months.

In summary, many new therapies are in development for the treatment of R/R aggressive NHL in patients who failed or are not eligible for SCT. However, at present, pixantrone is the only approved treatment in multiply-relapsed aggressive NHL.

Patients with Relapsed Diffuse Large B-Cell Lymphoma Who Fail Second-Line Salvage Therapy: Monotherapy Versus Combination Therapy

Professor Eric Van Den Neste

CORAL and SCHOLAR1 Studies

The CORAL study evaluated rituximab plus dexamethasone, cytarabine (high-dose Ara-C), and cisplatin (R-DHAP) versus R-ICE in patients with DLBCL.[18,21] Patients who were chemosensitive received carmustine, etoposide, cytarabine, and melphalan (BEAM) + ASCT and underwent a second randomisation to receive 1-year rituximab maintenance therapy or remain under observation. Overall, only about 50% of patients underwent SCT. An analysis was carried out on patients who failed this therapy, namely patients who failed to respond to R-ICE or R-DHAP (thus being ineligible for transplantation) in the first instance (refractory patients) and patients who relapsed immediately after transplantation or after the second randomisation.[22] In the refractory patients (i.e. patients who had failed R-ICE or R-DHAP), the overall response rate (ORR) to third-line chemotherapy (including switching regimens, anthracycline-containing regimens, and acute leukaemia-type treatment) was 39%, with a CR/CR unconfirmed (CRu) rate of 27%. There was no specific recommendation in the CORAL study regarding third-line treatments; frequently, the treatments entailed a switch from R-ICE to R-DHAP or vice versa. As no difference in results was observed between treatment types, it was concluded that there was no obvious cross-resistance between the R-ICE and R-DHAP regimens. Median overall survival for the entire population was 4.4 months; however, multivariate analysis revealed that the quality of response to third-line treatment significantly impacts the overall survival. Consequently, the median overall survival was 3.7 months in patients with stable disease/progressive disease, 11.7 months in the partial response group, and 63.6 months in the CR/CRu group. Similarly, patients who were eligible for ASCT (following

achievement of clinical response) demonstrated improved median survival versus patients ineligible for ASCT (11.1 versus 3.3 months).

With regard to patients who had relapsed post ASCT (n=75) where third-line treatment was at the discretion of the physician, the majority of patients switched between the R-DHAP and R-ICE regimens whenever possible. In these patients, the ORR was 44%, with a CR/CRu rate of 32%, and a small proportion of the patients, 16 of 75 (22%) patients, eventually underwent SCT.[22] In multivariate analysis, achievement of response, but not transplantation itself, was a very strong positive factor, together with the International Prognostic Index. In the case of relapse post transplantation, the interval between the CORAL transplantation and the time of relapse was important. These results demonstrate that it is still possible to achieve a response in a proportion of patients with chemorefractory DLBCL, allowing for ASCT in some and the possibility of longer-term survival.

A meta-analysis of the truly refractory patients (SCHOLAR1) was carried out whereby data from the CORAL study were combined with those from the MDACC, MC/IA, and LY.12 studies.[23] Eligible patients included those refractory to a first-line therapy (as defined by >4 cycles of R-CHOP-like regimens), refractory to a second-line therapy (>2 cycles of R-DHAP, R-ICE, or rituximab plus gemcitabine, dexamethasone, and cisplatin [R-GDP]), or with a relapse early (<12 months) after ASCT. A total of 529 of 635 chemorefractory patients could be evaluated for response. The ORR for the total population was 26%, with a CR rate of 8% and a partial response rate of 18%. These results emphasise the unsatisfactory results of current strategies in R/R DLBCL.

Table 1a: Selection of Phase II/III combination studies for relapsed/refractory aggressive non-Hodgkin lymphoma or diffuse large B-cell lymphoma.[18,24-31]

Regimen	Type of lymphoma	Number of patients (evaluable)	Median (range) number of prior lines	PFS, months	CR/CRu, %	ORR, %
R-bendamustine[24]	R/R DLBCL	61 (59)	1 (1-9)	3.6	15	46
R-bendamustine[25]	R/R DLBCL	63 (59)	1 (1-3)	6.7 at median 4.7 months follow-up	37	63
R-lenalidomide[26]	R/R DLBCL	17 (14)	3 (1-5)	38% at 2 years*	35	41
R-lenalidomide[27]	R/R DLBCL (elderly)	23 (23)	3 (2-8)	Estimated DFS: 34.8% at 1 year*	35	35
R-GEMOX[28]	R/R DLBCL	32 (30)	Mean 1.72, >1 regimen in 44%	29% at 12 months*	34	43
R-GEMOX[29]	R/R B-cell L	46 (46)	2 (1-5)	At 4 cycles, 2-year EFS: 43%	50	83
	DLBCL	33 (33)	NR	At 4 cycles, 2-year EFS: 42%	58	82
R-GEMOX[30]	R/R DLBCL	49 (48)	1; 74% one, 14% two, 12% refractory	5	44	61
GEMOX;[31] gemday 1, 8	R/R B-cell L	30 (30) [57% DLBCL]	2 (1-≥5)	OS 15 months	30	57
R-GEMOX;[31] gem day 1		32 (32) [50% DLBCL]	2 (1-4)	OS 24 months	50	78
R-ICE[18]	R/R DLBCL	197 (197)	1, all patients were in first relapse	3-years EFS: 26%	36	64
R-DHAP[18] (CORAL)		191 (191)		3-years EFS: 35%	40	63

*Percentage of patients.
CR: complete response; CRu: complete response unconfirmed; DFS: disease-free survival; DHAP: dexamethasone, cytarabine, cisplatin; DLBCL: diffuse large B-cell lymphoma; EFS: event-free survival; gem: gemcitabine; ICE: ifosfamide, carboplatin, etoposide; L: lymphoma; NHL: non-Hodgkin lymphoma; NR: not reported; ORR: overall response rate; OS: overall survival; PFS: progression-free survival; R: rituximab; R/R: relapsed/refractory; R-GEMOX: rituximab, gemcitabine, oxaliplatin.

Table 1b: Selection of Phase II/III monotherapy studies for relapsed/refractory non-Hodgkin lymphoma or diffuse large B-cell lymphoma ineligible for high-dose therapy.[32-39]

Regimen	Type of lymphoma	Number of patients (evaluable)	Median (range) number of prior lines	PFS/OS, months	CR/CRu, %	ORR, %
Gemcitabine[32]	R/R a-NHL	31 (30)	2 (1-3)	6 for responders/NR	0	20
Rituximab[33]	R/R a-NHL	21 (21)	2.5 (1->3)	3.8/8.6	4.8	38.1
Lenalidomide[34]	R/R a-NHL	217 (217)	3 (1-13)	3.7/NR	13	35
	R/R a-DLBCL subgroup	108 (108)	NR	2.7/NR	7	28
Lenalidomide[35]	R/R a-NHL	49 (49)	4 (1-≥5)	4.0/NR	12	35
Bendamustine[36]	R/R a-NHL	21 (18)	2 (1-4)	3.5/NR	17	44
Ibrutinib[37]	DLBCL	80 (80)	3 (1-7)	1.6/6.4	10	25
	ABC DLBCL	38 (38)	3 (1-7)	2.0/10.3	16	37
Bortezomib[38]	R/R NHL (excl. MCL)	21 (21)	4 (1-12)	36% at 6 months*	9.5	19
Oxaliplatin[39]	Relapsed/recurrent a-NHL	22 (22)	2 (1-≥4)	NR/NR	4.5	32

*Percentage of patients

ABC: activated B-cell; a-DLBCL: aggressive diffuse large B-cell lymphoma; a-NHL: aggressive non-Hodgkin lymphoma; CR: complete response; CRu: complete response (unconfirmed); DLBCL: diffuse large B-cell lymphoma; MCL: mantle cell lymphoma; NHL: non-Hodgkin lymphoma; NR: not reported; ORR: overall response rate; OS: overall survival; PFS: progression-free survival; R/R: relapsed/refractory.

Combination and Monotherapy Treatment for Relapsed/Refractory Aggressive B-Cell Non-Hodgkin Lymphoma or Diffuse Large B-Cell Lymphoma

Current combinations that are being studied in Phase II/III studies for R/R aggressive B-cell NHL or DLBCL include regimens such as rituximab (R)-bendamustine, R-gemcitabine, and oxaliplatin (R-GEMOX), R-lenalidomide, R-ICE, and R-DHAP. In a selection of studies (Table 1a), ORRs ranged from 35–83%, while CR/CRu rates ranged from 15–58%, demonstrating that there is a proportion of patients who can achieve a clinical response.

In a selection of Phase II/III studies assessing various monotherapies in patients with R/R NHL or DLBCL ineligible for high-dose therapy (Table 1b), ORRs ranged from 19–44%, while CR/CRu rates ranged from 0–17%. Despite the limitations of between-study comparisons (e.g. low patient numbers, mixed populations, and difference in median number of prior treatment lines), these results suggest that a small proportion of patients in this setting are still able to achieve complete remission.

In summary, achievement of a complete response in the setting of R/R lymphoma remains a positive prognostic factor. Polychemotherapy is usually preferred for transplant-eligible patients and monotherapies are usually reserved for other patients (with pixantrone being a good option with non-negligible efficacy). Currently awaited are the results of ongoing studies evaluating the incorporation of pixantrone into multiple-drug regimens and potentially as a bridge to transplantation.

Pixantrone for Third and Fourth-Line Treatment of Relapsed/Refractory Non-Hodgkin B-Cell Lymphoma

Professor Ruth Pettengell

Pixantrone: Indication and Guideline Recommendations

Pixantrone is the only drug that has evidence from Phase III randomised controlled trials (RCT) and is licensed as a single agent for the third and fourth-line treatment of multiple R/R-aggressive B-cell NHL within Europe. Conditional marketing authorisation was granted in the European Union (EU) (May 2012) with the specific obligation to conduct the PIX306 study, a Phase III RCT.[13] The use of pixantrone is mentioned in the European Society for Medical Oncology (ESMO) guidelines,[1] as well as in many national guidelines, including the National Institute for Health and Care Excellence (NICE) guidelines.[40]

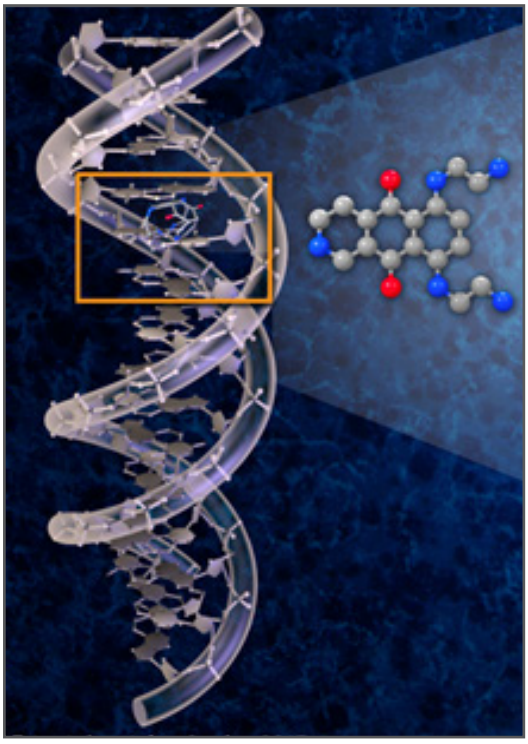
Mechanism of Action and Unique Chemical Structure of Pixantrone

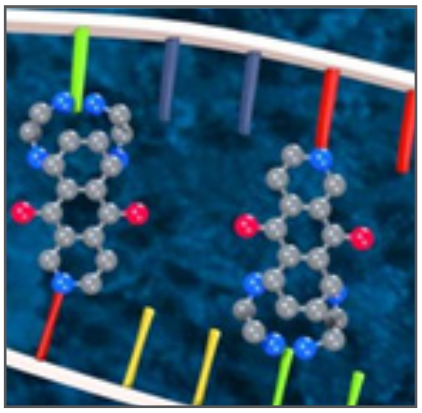
Pixantrone is a novel aza-anthracenedione with a mechanism of action that is distinct from that of anthracyclines and anthracenediones, such as doxorubicin and mitoxantrone. Classical anthracyclines work by inhibiting DNA topoisomerase 2 alpha, resulting in DNA damage and relaying a signal through the p53 cell cycle-dependent pathway, ultimately resulting in apoptosis. In contrast, pixantrone works predominantly as a DNA intercalator (Figure 2); the resultant chromosome bridges and micro and multi-nucleation eventually lead to abnormal mitosis and cell death.[41]

The chemical structure of pixantrone also confers unique properties in terms of safety. Compared with anthracyclines, pixantrone lacks an iron-binding site.[42,43] It therefore does not form toxic drug-metal complexes,[43] which limits the release of reactive oxygen species and concomitant cardiac toxicity. Additionally, alcohol metabolites of pixantrone accumulate to a lesser degree in cardiac tissue[14] compared with doxorubicin, and furthermore, pixantrone appears to be able to displace the previously accumulated doxorubicin alcohol metabolites. As such, pixantrone does not add to the existing toxicity in patients previously treated with anthracyclines. In summary, pixantrone lacks reduction–oxidation (i.e. redox) activity and inhibits doxorubicinol formation in human myocardium.[44]

Reduced Cardiotoxicity of Pixantrone in the Clinic Compared with Doxorubicin

Results from RCTs have demonstrated that the findings of preclinical studies have carried through to clinical practice. In the first trial, a comparison of a pixantrone-based regimen (R-CPOP: rituximab with cyclophosphamide, pixantrone, vincristine, and prednisolone) was compared with doxorubicin-based therapy (R-CHOP) for first-line therapy in DLBCL patients.[45] Compared with the R-CHOP arm, a lower percentage of R-CPOP-treated patients had a decrease from baseline in left ventricular ejection fraction (decreases of ≥10% to <50% [27% versus 15%, respectively]; ≥15% [32% versus 17%]; ≥20% [17.5% versus 2%]). In addition, a higher percentage of R-CHOP-treated patients had a significant increase in troponin T (33% versus 7%, respectively). These results demonstrate that indicators for cardiac toxicity are favourable for pixantrone even in the first-line setting. In terms of clinical evidence of cardiotoxicity, 6% of patients in the R-CHOP arm experienced Grade 3 congestive heart failure compared with none in the R-CPOP group.





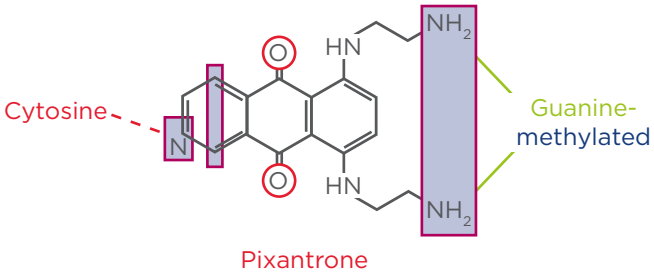


Figure 2: Pixantrone has a unique mechanism of action.
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PIX301 was a multicentre, randomised, active-controlled study evaluating the efficacy and safety of pixantrone as a single-agent therapy in the management of patients with aggressive R/R-NHL (73% with DLBCL) who had received at least two prior therapies and had adequate cardiac function (left ventricular ejection fraction: ≥50%) at time of study entry.[14] Patients were randomised 1:1 to either pixantrone (50 mg/m^2 on Days 1, 8, and 15, every 28 days, ≤6 cycles) or to available active comparators in the EU and USA (physician's choice, used at standard therapeutic doses and schedules). Patients had a maximum prior cumulative dose of anthracyclines of 450 mg/m^2 or equivalent, indicating that patients were heavily pretreated upon study entry. Importantly, patients had to have had at least a 6-month response to previous anthracycline-based chemotherapy and therefore this study excluded primary refractory patients. Baseline characteristics were well-balanced between the pixantrone and the comparator arm except for cardiac toxicity; 3 patients in the pixantrone group had a history of congestive heart failure and 2 had cardiomyopathy (no history of cardiac problems was reported in the comparator arm); hence, it is difficult to interpret the cardiac outcomes in the study.

A significant difference was observed between the pixantrone and comparator arms in terms of CR/CRu rates at the end of study (24% versus 7%, respectively); the median duration of CR/CRu (9.6 versus 4 months, respectively) and progression-free survival (5.3 versus 2.6 months, with a hazard ratio of 0.6).

In terms of pixantrone safety, the predominant toxicity (Grade 3/4) was haematological events, with neutropenia in particular (41% versus 19% in the comparator arm). However, this did not translate into a great increase in febrile neutropenia (7% versus 3% in the comparator arm). In this study, granulocyte colony-stimulating factor (G-CSF) use was allowed; however, as no specific guidelines on the use of G-CSF were provided in the study protocol, few patients received G-CSF as recommended. The rate of anaemia was higher in the comparator arm (13% versus 6% in the pixantrone arm) while thrombocytopenia rates were comparable in both arms (12% and 10% in the pixantrone and comparator arms, respectively). However, it is difficult to make comparisons with the comparator group as the median number of drug cycles was different in the pixantrone and comparator arms (four versus three cycles, respectively), and there was a discrepancy in the frequency of blood tests between the two groups due to the specific treatment administration schedule in the pixantrone arm. In terms of non-haematological toxicity, rates of events were low. Cardiac events were observed in the pixantrone group but as mentioned above, interpretation of these results is difficult due to the imbalance in cardiac abnormalities at baseline; nonetheless, the majority of the cardiac events were asymptomatic and reversible, having resolved by the end of the study.

PIX306 Study

A post hoc analysis of the PIX301 study showed that the efficacy of pixantrone versus comparator was independent of prior rituximab therapy.[15] Pixantrone has conditional marketing approval in the EU based on the results of the PIX301 study.[13] The PIX306 study design was endorsed by the EMA as a post-marketing commitment required to convert to full marketing approval. The study aimed to confirm the efficacy of pixantrone in patients progressing after prior rituximab-containing regimens, as well as to provide information to guide treatment of transplant-ineligible relapsed lymphoma patients. Gemcitabine is used in the comparator arm (in combination with rituximab), as it was commonly in use as a single agent/doublet at the time of the study design. Additionally (unlike PIX301), patients only had to have had a 12-week response to a previous line of therapy and there were no specific requirements in terms of prior anthracycline use. To date, 291 (out of a planned recruitment of 320) patients have been recruited and preliminary results are expected in summer 2018.

Patient Cases

Doctor Amjad Hayat and Professor Kai Hübel

Dr Hayat and Prof Hübel presented case studies of patients with relapsed DLBCL who received pixantrone monotherapy. The cases demonstrated that treatment with pixantrone is manageable in older patients, and is an option in heavily pretreated patients not responding to chemotherapy.

PANEL DISCUSSION

Q: With the availability of a vast range of novel treatments for R/R DLBCL, how do you choose which to use and which patients should receive these treatments? Please comment on the toxicity

profile of pixantrone in comparison with some of the novel agents.

Prof Zinzani replied that, for the most part, the novel agents are still in clinical trials; generally, pixantrone was prescribed after the second-line treatment for patients who were ineligible for SCT, due to lack of cardiac toxicity and the higher clinical response compared with other biological agents.

The major toxicities with pixantrone are neutropenia and cytopenia; however, these toxicities could be managed by limiting the dosing schedule to Days 1 and 8 (and not Day 15). Satisfactory (clinical) results were obtained with this change in schedule.

Q: How is neutropenia managed in response to chemotherapy and is neutropenia a big concern?

Prof Van Den Neste replied that one of the major considerations when choosing a treatment regimen is the rate of Grade 3/4 neutropenia, as well as the rates of infection, infestation, and febrile neutropenia. Another consideration is whether the neutropenia can be treated with growth factors (GFs) and whether these GFs are available/reimbursable. Pixantrone had a relatively favourable profile, even though it is associated with significant haematological toxicities, because of the low rate of infection. The issue would be how to give GFs in combination with weekly pixantrone infusions (unlike DHAP or ICE).

Prof Pettengell added that it would be important for GFs to be started within 72 hours of chemotherapy and ideally stopped 24 hours before the next cytotoxic chemotherapy. She also mentioned that she would be comfortable treating with G-CSF between pixantrone injections, as it was not a cell-cycle specific agent.

Q: Which patients in your practice would you choose to treat with pixantrone?

Prof Van Den Neste replied that he would select patients that were 'in between', meaning patients who were not eligible or had relapsed after ASCT, but who were still able to cope with frequent hospital visits, had the need for antibiotics in case of neutropenia, as well as being able to cope with febrile neutropenia. As haematological adverse events are an issue, the patients would still have to be selected with caution (perhaps with the use of comorbidity scoring methods).

SUMMARY AND CONCLUSION

Within clinical practice, a significant proportion (about 75%) of patients with R/R aggressive B-cell NHL who fail first-line therapy will go on to potentially having third or fourth-line therapy. There is no accepted standard of care for third or fourth-line treatment. Pixantrone monotherapy has significantly greater efficacy than active comparator agents in these patients and can be used for patients with significant prior anthracycline exposure. It has a predictable and manageable safety profile, and in addition, a flexible dosing schedule can be utilised to manage toxicities. Clinical trials of pixantrone combined with other standard regimens are ongoing.

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REDEFINING LATER-LINE THERAPY IN METASTATIC COLORECTAL CANCER

This symposium took place on 29th June 2017 as part of the 19th World Congress on Gastrointestinal Cancer in Barcelona, Spain

Chairpersons

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Speakers

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MEETING SUMMARY

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer death in the world, accounting for approximately 1.4 million new cases and almost 700,000 deaths in 2012.[1] The objective of the symposium was to provide an overview of the current treatment landscape in terms of later-line therapy in metastatic CRC (mCRC) and to discuss the evidence for the various options available, including rechallenge and therapies such as trifluridine (FTD)/tipiracil (TPI) (Lonsurf®; also known as TAS-102) and regorafenib (Stivarga®). The symposium started by examining the clinical value of third-line treatment in patients with mCRC and providing an insight into the mechanism of action of FTD/TPI, and a comparison with that of 5-fluorouracil (5-FU). The safety and efficacy of FTD/TPI was then discussed together with the practical management of patients on treatment. The speakers tackled the issue of rechallenge and reintroduction as an option in the third-line, reviewing the pros and cons, and the available studies providing information on the safety and efficacy of the different options in later lines, concluding that there is a lack of robust evidence for rechallenge as a clinical decision. This was followed by a review of the compelling evidence for the use of treatments such as FTD/TPI and regorafenib in the third-line, with documented evidence for efficacy.

Welcome and Introduction

Professor Dirk Arnold

According to the 2016 European Society for Medical Oncology (ESMO) guidelines for the treatment of mCRC, first and second-line treatments comprise mainly a combination of chemotherapy (e.g. fluoropyrimidines with oxaliplatin and/or irinotecan) and monoclonal antibodies ([mAb]; e.g. bevacizumab, cetuximab, panitumumab, ramucirumab) or aflibercept.[2] In recent years, the proportion of patients who are candidates for treatment beyond the second-line, i.e. fit enough to receive treatment, according to the current ESMO guidelines, has increased to 50–60%.[2] This is likely due to the improvements in earlier treatment lines, as more and more patients remain in good performance status with controlled disease conditions. Treatment options beyond second-line include single agents such as FTD/TPI and regorafenib, as well as mAb and combination

therapy for patients who have not previously received an anti-epidermal growth factor receptor (EGFR) antibody. The challenge is how to determine the appropriate treatment for use given the options available.

CLINICAL VALUE OF THIRD-LINE TREATMENTS IN PATIENTS WITH METASTATIC COLORECTAL CANCER

Understanding the Added Value of Trifluridine/Tipiracil

Professor Andres Cervantes

FTD/TPI is indicated for the treatment of adult patients with mCRC who have been previously treated with, or are not considered candidates for, available therapies including fluoropyrimidine, oxaliplatin, and irinotecan-based chemotherapies, anti-vascular endothelial growth factor agents, and anti-EGFR agents.[3] It is currently approved in the USA, the European Union (EU), Japan, and Argentina.[3-6] The use of FTD/TPI in the third-line setting in mCRC is recommended in national and international guidelines such as ESMO, National Comprehensive Cancer Network (NCCN), National Institute for Health and Care Excellence (NICE), and the Japanese Society for Cancer of the Colon and Rectum (JSCCR).[2,5,7,8]

Mechanism of Action of Trifluridine/Tipiracil

FTD/TPI is a novel oral antitumour nucleoside; it is made up of FTD, a thymidine-based nucleoside analogue, and TPI, a thymidine phosphorylase inhibitor, at a molar ratio of 1:0.5. FTD exerts its cytotoxic effects primarily by incorporation of its active metabolite, FTD triphosphate (trifluoromethyl deoxyuridine 5'-triphosphate [F_3dTTP]), into DNA.[9-11]

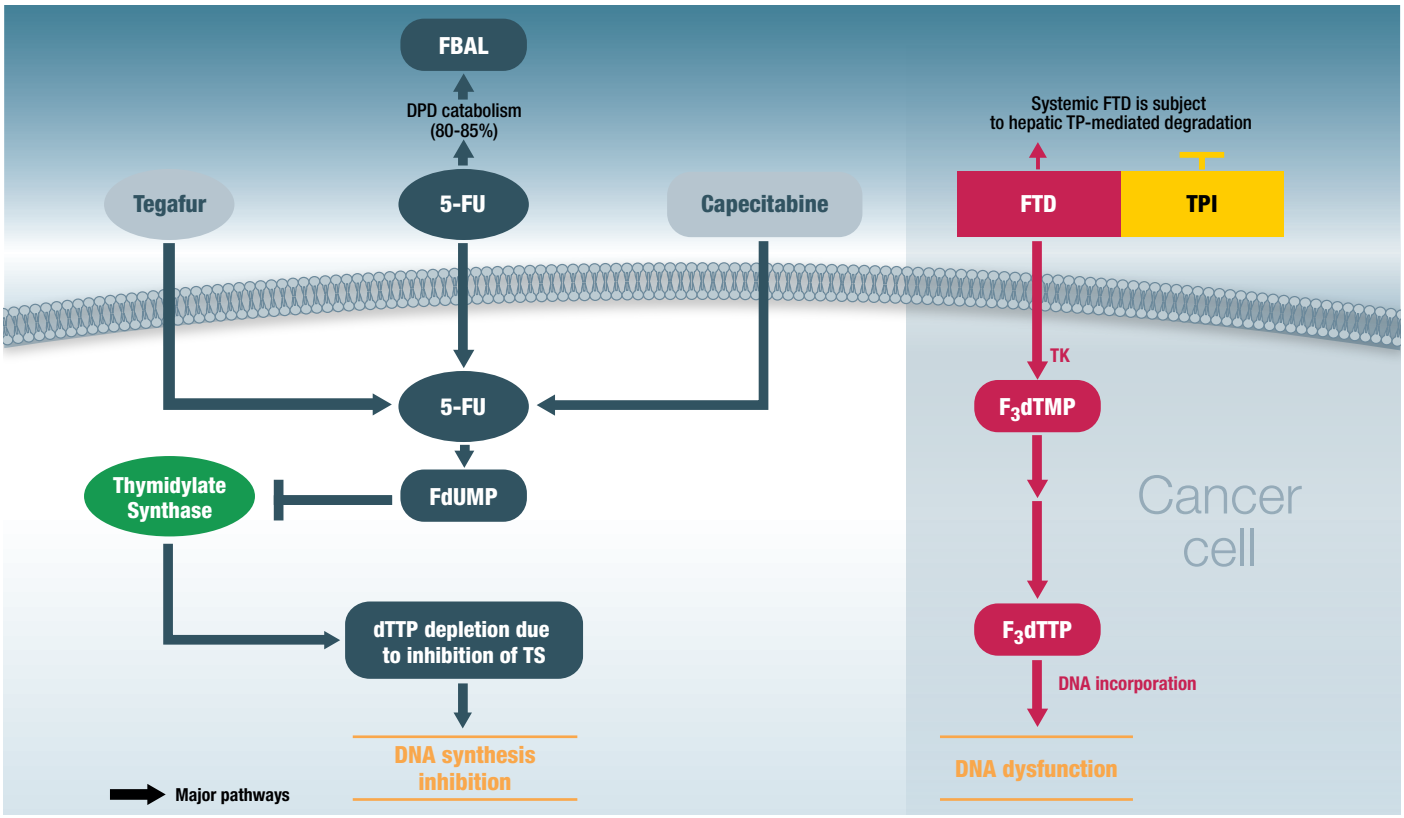

Figure 1: Comparison of mechanisms of action of trifluridine/tipiracil (right-hand panel) versus 5-FU (left-hand panel).[9,13]

5-FU: 5-fluorouracil; DPD: dihydropyrimidine dehydrogenase; dTTP: deoxythymidine triphosphate; FBAL: alpha-fluoro-beta-alanine; F_3dTMP: trifluoromethyl deoxyuridine 5'-monophosphate; F_3dTTP: trifluoromethyl deoxyuridine 5'-triphosphate; FdUMP: fluorodeoxyuridine monophosphate; FTD: trifluorothymidine (trifluridine); TK: thymidine kinase; TP: thymidine phosphorylase; TPI: tipiracil hydrochloride; TS: thymidylate synthase.

Under normal circumstances, systemic FTD is subject to hepatic, thymidine phosphorylase-mediated degradation; co-administration of TPI inhibits thymidine phosphorylase, thus increasing the bioavailability of FTD (37-fold increase in exposure) for conversion to the active metabolite within cells.[9,12]

Entry of FTD into the cancer cell (via active transportation or nucleoside transporters) is followed by phosphorylation by thymidine kinase to ultimately produce F_3dTTP (Figure 1). F_3dTTP is readily incorporated into the DNA of tumour cells (in the place of thymidine bases), thus interfering with DNA function and inhibiting cell proliferation and tumour growth.[9] The mechanism of action of FTD is in contrast with that of 5-FU, a uracil-based nucleic acid analogue, which exerts its antitumour activity by inhibition of DNA synthesis via depletion of deoxythymidine triphosphate (dTTP) (due to inhibition of thymidylate synthase) (Figure 1).[9]

Interestingly, in preclinical studies, FTD/TPI demonstrated antitumour activity in 5-FU-sensitive as well as 5-FU-resistant cells.[14] This observation was carried through in the RECOURSE study, which demonstrated that FTD/TPI was also clinically active in patients who are refractory to 5-FU.[15] In summary, DNA dysfunction by FTD/TPI is distinct from the mechanism of action of 5-FU, resulting in antitumour activity of FTD/TPI in both 5-FU-sensitive and resistant tumours.

Clinical Efficacy and Safety of Trifluridine/Tipiracil

Professor Marc Peeters

The median overall survival (OS) for patients with mCRC is currently >30 months, as a result of the availability of new drugs as well as the multidisciplinary approach to the continuum of care.[2] Moving from first-line to later treatment lines, it is important to achieve a balance, not just between the efficacy and safety of treatments, but also the quality of life provided by these treatments. In the third-line setting, two drugs (FTD/TPI and regorafenib) have demonstrated efficacy versus best supportive care (BSC) in heavily pretreated patients with mCRC in randomised Phase III trials. However, no head-to-head data for these two options are available.

Data from three clinical trials are available for FTD/TPI: a Phase II trial (J003, N=172),[16] the Phase III RECOURSE trial (N=800),[15] and the Phase III TERRA trial, which was an East Asian specific study (N=406).[17] The participants in these trials were patients with refractory disease having previously received ≥2 regimens prior to study entry and with Eastern Cooperative Oncology Group (ECOG) performance status of 0–1 in the majority of patients. The patients were randomised in a 2:1 ratio of FTD/TPI with BSC versus placebo with BSC. Patients randomised to FTD/TPI received a dose of 35 mg/m^2 twice daily on Day 1 up to Day 5, and then from Day 8 until Day 12, every 4 weeks.

Overall, the three studies showed the following with regard to efficacy:[15-17]

- Significant and clinically meaningful improvements in median OS were observed with FTD/TPI versus placebo, with hazard ratios (HR) ranging from 0.56–0.79.[15-17] In the RECOURSE study, the OS benefits of FTD/TPI were observed not only in the overall population but also across different patient subgroups.[15]
- FTD/TPI offered a significant and clinically meaningful improvement in progression-free survival (PFS) compared with placebo, with HR ranging from 0.41–0.48;[15-17] PFS benefits were also consistent across all prespecified subgroups in the J003 and RECOURSE trials.[15,16]
- FTD/TPI offered consistent improvements in disease control rate compared with placebo (about 44% versus about 15%).[15-17]
- FTD/TPI effectively prolonged time to deterioration of ECOG performance status from 0–1 to ≥2 (5.7 versus 4.0 months for placebo) in the RECOURSE study.[15]
  - 84% of patients remained at ECOG performance status 0–1 at treatment discontinuation.[18]

With regard to safety, the RECOURSE study showed the following:[15]

- FTD/TPI had a well-tolerated safety profile with a low rate of dose reductions, discontinuations, and severe adverse events (AEs).
- Overall, only 4% of patients on FTD/TPI withdrew due to AEs (versus 2% for placebo), while 14% required a dose reduction.
- Treatment with FTD/TPI was well tolerated with minimal non-haematological AEs (with the most common being nausea/vomiting, decreased appetite, fatigue, and diarrhoea).
- The main AEs associated with FTD/TPI were haematological in nature (neutropenia, leukopenia, and anaemia), which were generally

manageable; guidelines are available for the management of these AEs.[3]

These safety results were consistent with what has been observed in the other two trials.[16,17]

Appropriate dose management is important for optimal clinical efficacy. With regard to haematological events, complete blood cell counts must be obtained prior to initiation of each cycle. In the event of neutropenia and thrombocytopenia, treatment with FTD/TPI should be interrupted and resumed only on recovery of neutrophil counts to ≥1.5 × 10^9/L and platelets to ≥75 × 10^9/L, respectively. Details for the management of neutropenia are provided in Figure 2. Similar guidance is recommended with regard to the management of Grade 2/3 and Grade 4 thrombocytopenia.

With regard to non-haematological AEs, the recommendation for most Grade 3 or 4 events would be to interrupt treatment and re-initiate with a dose 5 mg/m^2 lower than the previous dose when the AE is resolved (to baseline levels). The exceptions to this recommendation are Grade 3/4 nausea and/or vomiting events that can be controlled by antiemetic therapy, and diarrhoea that can be managed with antidiarrhoeal medicinal products. In the majority of cases, it may not be necessary to decrease the dose of FTD/TPI.

These recommendations are in line with the observation that only 4% discontinuation due to AEs were reported in the RECOURSE trial, which demonstrates the effectiveness of management of AEs with dose delay and reduction strategies. In summary, FTD/TPI is effective as a treatment option for mCRC beyond second-line, with a favourable safety profile.

TREATMENT CHOICE AT THIRD-LINE: TO RECHALLENGE OR NOT TO RECHALLENGE?

Rechallenge in the Continuum of Care

Professor Dirk Arnold

According to the 2016 ESMO guidelines, there is an option of the usage of either new single agents, or a rechallenge or reintroduction of treatment of mCRC beyond the second-line.[2,19] Rechallenge refers to the reinitiation of a therapy (after an intervening period) to which the tumour had proved to be resistant in earlier-line treatments.[19]

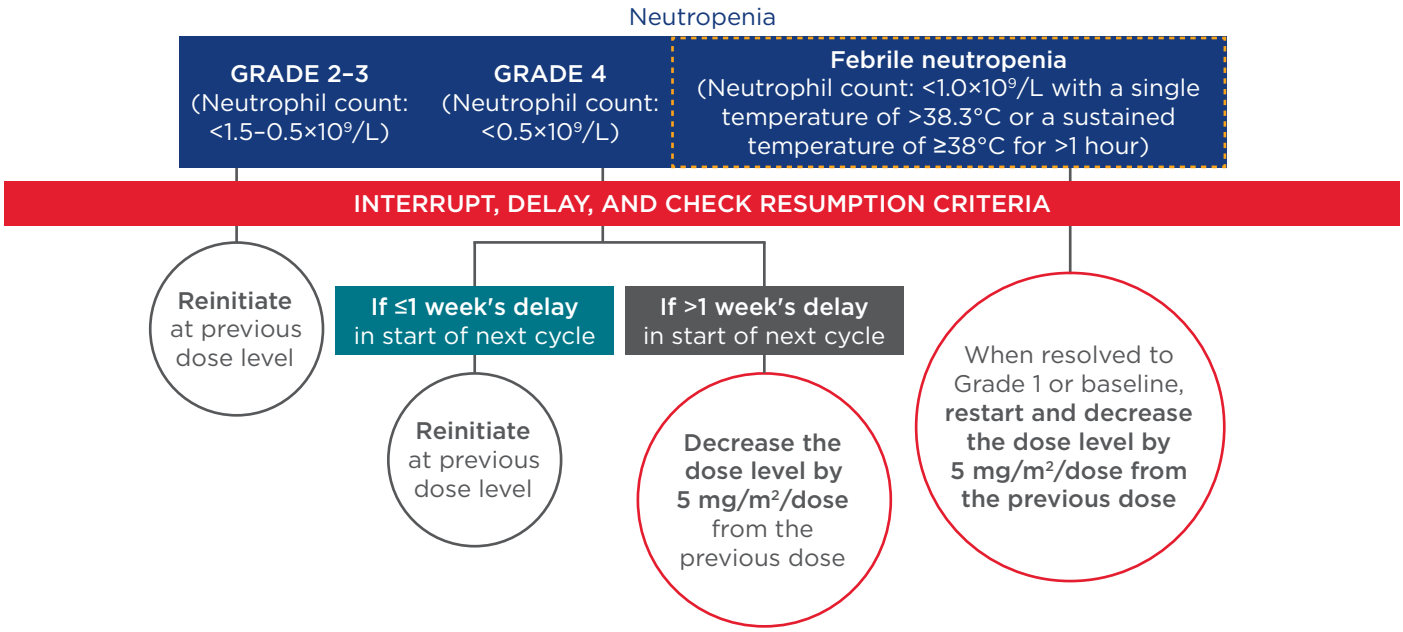

Figure 2: Recommendations for dose delay and dose reduction for patients with neutropenia.[3]
Resumption criteria (CTCAE, Grade 1 or better): neutrophils, ≥1.5 × 10^9/L; platelets, ≥75 × 10^9/L.
Resumption criteria applied at the start of the next cycle for all patients regardless of whether or not the interruption criteria were met.
CTCAE: Common Terminology Criteria for Adverse Events.

By contrast, reintroduction refers to the reinitiation of treatment without evidence of disease progression in the interval; this may be according to a set duration (e.g. adjuvant treatment) or following a planned treatment break (e.g. for the reduction or management of AEs).

The rationale for rechallenge lies in the observation that cancer is a clonal disease and that tumour progression is generally not homogeneous. It is feasible that some clones that were sensitive to first-line treatment, but had escaped elimination, would re-emerge as clones that would be the source of tumours that progress following second-line treatment. It stands to reason that the first-line treatment may therefore have a clinical effect on the tumour in the third-line setting.

In the OPTIMOX studies, previously untreated patients were randomly assigned to either a combination of leucovorin (LV) and fluorouracil (FU) with oxaliplatin (FOLFOX4) administered every 2 weeks until progression, or a simplified LV and FU regimen with high-dose oxaliplatin (FOLFOX7) for six cycles, maintenance without oxaliplatin for 12 cycles, and reintroduction of FOLFOX7. The pooled analysis of OPTIMOX-1 and OPTIMOX-2 suggests that reintroduction after a ≥6-month oxaliplatin-free interval improves PFS, OS, and response rates in treatment-naïve patients with mCRC.[20] Similar results were also observed in more complex treatment regimens such as LV and FU and oxaliplatin and irinotecan (FOLFOXIRI) treatment, with response rates of 38% following rechallenge in patients who have progressed following maintenance treatment.[21]

A similar observation was also noted for cetuximab rechallenge in patients with irinotecan-refractory mCRC who had a clinical benefit after initial cetuximab plus irinotecan therapy but experienced disease progression following treatment with both cetuximab and a second-line of chemotherapy. Rechallenge resulted in an overall response rate of ≤54%.[22] Molecular analyses of circulating tumour cells and circulating tumour DNA have demonstrated the reappearance of anti-EGFR-sensitive tumour cell clones after ceasing targeted therapy,[23] suggesting that rechallenge with anti-EGFR agents may be a viable option for these patients.

Nonetheless, examination of the available literature on rechallenge with anti-EGFR in mCRC indicates limited evidence for this option, as the majority of studies are retrospective in nature with poor levels of evidence (Level IV).[22,24-26] A similar situation is observed with the concept of retreatment with oxaliplatin, with poor evidence levels for the available studies. In summary, reintroduction of treatment can produce a high overall response rate and may represent a viable option in certain circumstances.[21] While the mechanisms of action supporting rechallenge are not completely understood and the levels of evidence are poor, oxaliplatin rechallenge appears to be a viable option in mCRC (with oxaliplatin-free interval ≥6 months).[20]

Third-line Treatment in the Continuum of Care

Professor Julien Taieb

While the treatment goals for mCRC vary according to the different lines of systemic treatment, the treatment goal for patients receiving third-line treatment and beyond is the maintenance of good quality of life and performance status (given their short life expectancy). Indeed, treatment-related factors (toxicity) take on a more important role (compared with tumour/disease-related characteristics and patient-related factors) as a patient progresses through the continuum of care.

It appears that the evidence of rechallenge with chemotherapy and targeted agents is conflicting; very little data are available on irinotecan and there are no data on bevacizumb beyond the second-line. Evidence for oxaliplatin comprises mostly of reports on a first-line stop-and-go (intermittent) treatment strategy (FOLFIRI) rather than third-line rechallenge. A study comparing patients receiving interval chemotherapy between the first FOLFOX and second FOLFOX therapy or having a chemotherapy holiday demonstrated PFS and OS of 27 and 58 weeks, respectively, after a chemotherapy holiday, compared with 11 and 36 weeks, respectively, after interval chemotherapy.[27] These results demonstrate that clinical outcomes are worse with rechallenge compared with reintroduction of oxaliplatin.

Similarly, the evidence for rechallenge with anti-EGFR mAbs is conflicting. On the one hand, results of a Phase II, prospective study of *KRAS* wild type patients (N=39) with mCRC show that rechallenge with cetuximab plus irinotecan may achieve clinical benefit (objective response rate:

53.8% [complete response: 5.1%; partial response: 48.7%]) and delay disease progression in patients who had previously experienced clinical benefit.[19,22] On the other hand, panitumumab monotherapy has shown minimal benefit in patients with *KRAS* wild type mCRC (N=20) that has progressed on prior cetuximab.[28] In addition, it has been shown that response to rechallenge with anti-EGFR is influenced by the response to the first use as well as the interval between the first and second anti-EGFR treatment; prior responders with longer interval length were more likely to respond to anti-EGFR.[29] Finally, it is well known that *RAS* mutations are present in >50% of all mCRC, which would preclude the use of anti-EGFRs for rechallenge.[29]

In this situation, options that are available for patients ineligible for anti-EGFR rechallenge are the single agents regorafenib and FTD/TPI. The CORRECT study demonstrated the clinical benefits of regorafenib on OS (HR: 0.77; 95% confidence interval [CI]: 0.64–0.94; p=0.0052) and PFS (HR: 0.49; 95% CI: 0.42–0.58; p<0.0001) compared with placebo in third-line treatment of patients with mCRC.[30] The RECOURSE study demonstrated significant efficacy benefits with FTD/TPI on OS (HR: 0.68; 95% CI: 0.58–0.81; p<0.001, with a 2-month improvement in median OS), and PFS (HR: 0.48; 95% CI: 0.41–0.57; p<0.001) versus placebo.[15] It should be noted that treatment with FTD/TPI is not considered as a 5-FU rechallenge, as the mechanisms of action of the individual treatments are different (Figure 1).[9] This has been confirmed in the RECOURSE study, which demonstrated the clinical effects of FTD/TPI in patients who had progressed on 5-FU.[15] In particular, treatment with FTD/TPI prolonged the PS of patients compared with placebo, with almost 9 out of 10 patients retaining a PS of 0–1 at the end of the study.[15]

Currently, the ESMO 2016 consensus guidelines recommend that treatment be given at third-line followed by a rechallenge (but not conversely).[2] These recommendations for treatment with regorafenib or FTD/TPI in the third-line setting (ahead of rechallenge) are supported by the strong evidence for the clinical efficacy of regorafenib[18,30,31] and FTD/TPI[15-17] in prospective clinical trials in the third-line setting. In summary, evidence-based treatments that are recommended in national and international guidelines should be the preferred third-line treatment options.[2,7]

Summary and Conclusion

Professor Andres Cervantes

The main points of the symposium are summarised in Figure 3.

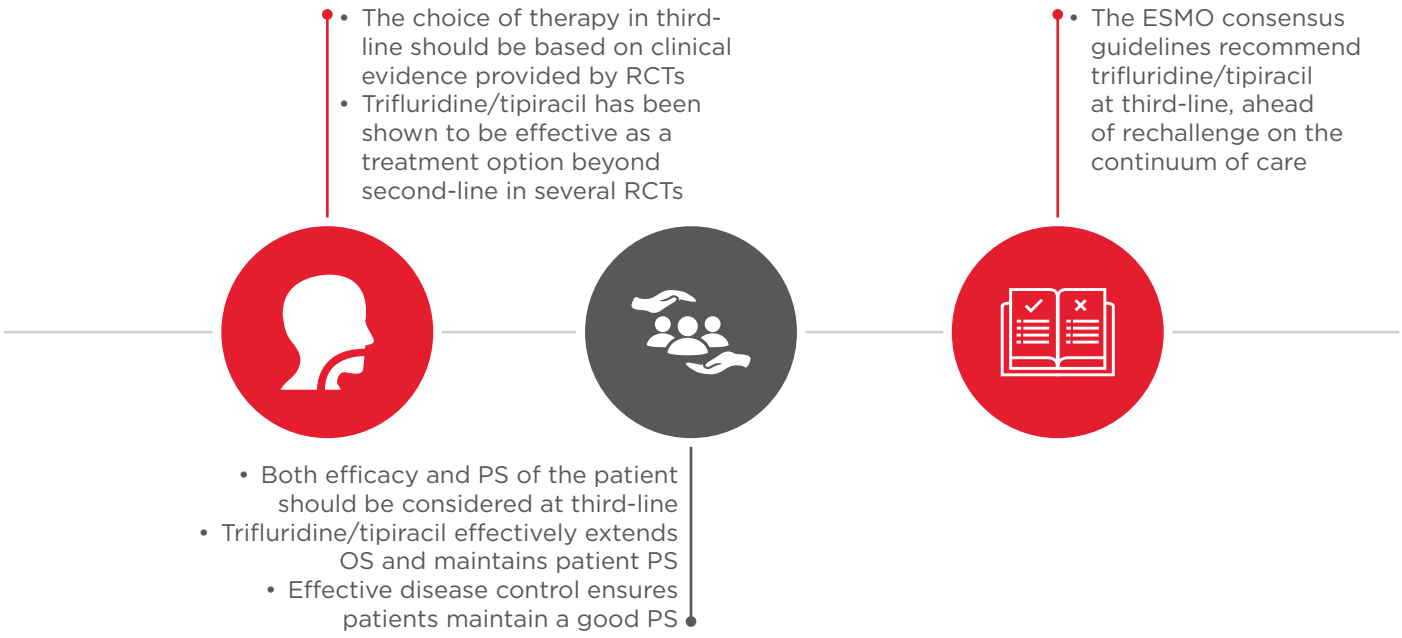

Figure 3: Summary of symposium.

ESMO: European Society for Medical Oncology; OS: overall survival; PS: performance status; RCT: randomised clinical trial.

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MANAGEMENT OF B-CELL LYMPHOMA: WHERE ARE WE NOW AND WHERE ARE WE GOING?

This symposium took place on 15th June 2017, as part of the 14th International Conference on Malignant Lymphoma (ICML) in Lugano, Switzerland

Chairperson
Gilles Salles[1]
Speakers
Nathan H. Fowler,[2] Umberto Vitolo[3]

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Disclosure: Prof Salles reports personal fees for consultancy, participation in symposia, or participation in advisory boards from Amgen, Bristol-Myers Squibb, Celgene, Gilead, Janssen, Kite, Merck, Morphosys, Novartis, Roche, and Servier, and has received a research grant from Roche. Prof Fowler reports grants from Celgene, Roche, and Abbvie, and personal fees from Celgene, Roche, Gilead, and Abbvie. Prof Vitolo reports receiving honoraria for participation in advisory boards for Roche, Janssen, and Celgene, and lecture fees from Roche, Celgene, Janssen, Gilead, and Takeda.
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MEETING SUMMARY

The main objectives of the symposium were to explore the current developments in the diagnosis and treatment of non-Hodgkin lymphoma (NHL). An overview of the hurdles and unmet needs in the management of indolent NHL were discussed, followed by the current and future perspectives for the treatment of indolent NHL. The topic of frontline treatment outcomes in diffuse large B-cell lymphoma (DLBCL), the most common type of high-grade NHL, was also explored with an emphasis on how outcomes could be improved.

Hurdles and Unmet Needs in the Management of Indolent Non-Hodgkin Lymphoma

Professor Gilles Salles

Prof Salles reviewed the current clinical management of indolent NHL, including epidemiological data, disease biology, heterogeneity, and progression. Learnings from these findings provided insights into current treatment goals.

The incidence of follicular lymphoma (FL) has almost doubled from the 1970s to the 21st century.[1] A survey conducted by the large collaborative group, InterLymph, revealed a number of FL risk factors.[1] An increase in FL risk was reported for individuals who had a first-degree relative with NHL (odds ratio [OR]: 1.99); had a greater BMI as a young adult (OR: 1.15); worked as spray painters (OR: 2.66); or had Sjögren's syndrome (OR: 3.37). Conversely, FL risk was reduced in individuals who had asthma, hay fever, or food allergy (OR: 0.79–0.85); had undergone blood transfusions (OR: 0.78); or had high recreational sun exposure (OR: 0.74).[1] At the genetic level, a strong association was found between high levels of t(14;18) translocation in prediagnostic blood samples from apparently

healthy individuals and development of FL; while there was no absolute threshold for the level of t(14;18) translocation, individuals with t(14;18) frequency reaching one in every 10,000 blood cells had a 23-fold greater risk of progression to FL.[2]

In FL, cells carry abnormalities which develop over time and lead to worse outcomes for overall survival (OS) with age.[3] In patients that relapse, analyses of clonal populations previously present in tumour nodes or serum were found to have diverged over time, emphasising the complexity of clonal evolution (Table 1).[4,5]

Gene expression analyses have highlighted the prognostic role of the tumour microenvironment, drawing correlations between increased macrophage count with worse outcomes;[6] these results were corroborated in a second independent study,[7] and partly reproduced by some[8,9] but not other studies.[10-12] Discrepancies in the latter findings may be attributed to changes within the microenvironment governed by patient population and treatment regimen. In patients with FL treated with rituximab plus combination chemotherapy of cyclophosphamide, adriamycin, vincristine, and prednisone (R-CHOP), the association between macrophage content and prognosis was reversed.[13]

In an effort to improve clinical indexes by mutational analysis or gene expression profiling, a team of German and Canadian investigators grouped seven mutated genes (*EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP,* and *CARD11*) and analysed them together with clinical factors in a model termed m7-FLIPI and refined this index by monitoring progression of disease (POD) at 24 months.[14] Based on mutation clusters, patients were classed into three risk categories: high, intermediate, and low. The m7-FLIPI model was correlated with survival probability and showed that patients in the high-risk group had worse 5-year failure-free survival versus low-risk patients (38.29% versus 77.21%; hazard ratio [HR]: 4.14; 95% confidence interval [CI]: 2.47–6.93; p<0.0001).[14] OS was also significantly worse in patients classified as high-risk by the model compared with low-risk patients. These results were echoed in a recent study showing that, when assessed at the 5-year time point, patients who failed primary therapy within 2 years after diagnosis (study cohort) had a 50% probability of survival compared with about 90% in the reference group.[15] Furthermore, re-treatment in these patients resulted in lower response rates and shorter response durations.[16]

In the PRIMA study, biopsies were assessed for histologic transformation (HT), an adverse event (AE) in the natural history of lymphoma, at first POD in FL patients who previously responded to immunochemotherapy. Almost 80% of patient biopsies had FL histology, of which 20% had HT. POD with HT appeared to occur early, with a median relapse time of 10 versus 23 months in populations with and without HT, respectively.[17] Clonal variation and clonal prevalence were examined from cellular biopsies of patients during relapse or during transformation. In patients without a transformation, clonal populations, although varied over time, were made up of the same few clones over the evolution of lymphoma. In patients with a HT, however, new clones emerged over time that were not detected at diagnosis.[18] Earlier relapse may increase the likelihood of transformation, but at present we lack the molecular tools at least using mutational analysis to predict transformation. Current treatments provide some benefit to patients, but are also linked with a range of side effects associated with chemotherapy, including fatigue, alopecia, development of neuropathy, and heart failure. Patients may also be at an increased risk of developing secondary AML, myelodysplastic syndrome, or potentially, secondary tumours.[19,20]

Current treatments enable us to treat most tumours, but it becomes increasingly difficult when patients experience HT. Therefore, finding an ideal therapeutic target for the treatment of FL remains to be identified. To address this issue, newer therapies may need to target self-renewing cancer progenitor cells to halt FL progression and transformation. Of interest is that the presence of the lymphoma-associated t(14;18) translocation in B cells can appear many years before disease manifestation in FL and confers a survival advantage for the mutated B cell by preventing apoptotic death. However, this translocation alone appears to be insufficient to drive development of FL and must be followed by a secondary oncogenic event. How these cells traffic through germinal centres and receive antigenic triggers to evolve to FL precursor cells is a question of major interest. There may be secondary events leading to the generation of cancer progenitor cells, which may proliferate and give rise to tumours. We may have the tools to treat tumours, but this becomes increasingly difficult after tumour transformation. What these data show is that there is a need to target these precursor cells to eliminate tumours in the hope of curing patients.

Table 1: Hurdles and unmet needs in follicular lymphoma.

Individual differences	Factors influencing incidence and prognosis
Biological heterogeneity	• Age, weight, genetic mutations
Clonal evolution and the tumour microenvironment	• Selective pressures of inherent mutations and the associated microenvironment • Cancer progenitor cells • Direct clonal evolution or divergent evolution from a common progenitor cell • Histologic transformation

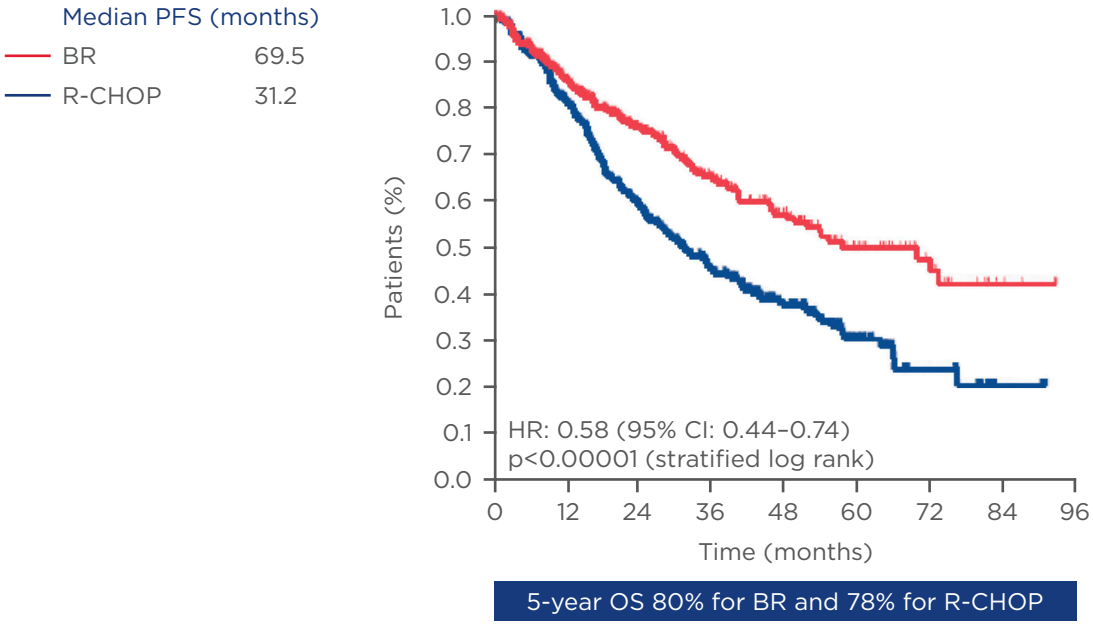


Figure 1: Progression-free survival in patients with indolent and mantle-cell lymphomas on BR versus R-CHOP as first-line treatment.

BR: bendamustine, rituximab; CI: confidence interval; HR: hazard ratio; OS: overall survival; PFS: progression-free survival; R-CHOP: rituximab plus cyclophosphamide, adriamycin, vincristine, and prednisone.

Progress and Promise: Current and Future Perspectives on Therapy of Indolent Non-Hodgkin Lymphoma

Professor Nathan H. Fowler

Clinical trials in FL have progressed over the last 30 years, from treatment regimens containing bleomycin, interferon, and very intensive regimens of alternating triple therapy, to treatments such as rituximab plus fludarabine, mitoxantrone, and dexamethasone. Although OS rates improved with advances in treatment regimens, improvements in failure-free survival were modest, and most patients still relapsed in the first several years following induction therapy.[21]

One of the first randomised studies to investigate the most effective chemotherapy regimen to combine with rituximab for first-line therapy of advanced FL was the FOLL-05 trial in 2013.[22] The study highlighted that rituximab was commonly used for patients with advanced FL, but there was no clear optimal associated chemotherapy regimen. In this landmark study, 500 treatment-naïve patients with 'active' FL were randomised (1:1:1) to rituximab plus cyclophosphamide, vincristine, prednisone (R-CVP); R-CHOP, or fludarabine, mitoxantrone (FM).[22] R-CHOP and R-FM were found to be superior to R-CVP in progression-free survival (PFS), but not OS, which was similar across treatment arms.[22,23] However, the FM group had higher rates of infection and cytopenia, and increased risk of secondary cancers.[22] R-CHOP offered the longest disease control, with better overall outcomes compared with R-FM or R-CVP. In the same year, the StiL NHL1 study reported R-CHOP treatment versus the bendamustine, rituximab (BR) regimen in patients with newly

diagnosed Stage III or IV indolent or mantle-cell lymphoma, with the primary objective of assessing non-inferiority of BR versus R-CHOP for PFS.[24] BR demonstrated superiority over R-CHOP for PFS (31.2 versus 69.5 months; p<0.00001), but not for OS over a 5-year (Figure 1) or 10-year period.[24,25]

In the 2014 BRIGHT study, BR was found to be non-inferior to R-CVP or R-CHOP for complete response rate when assessed in treatment-naïve patients with indolent NHL or mantle cell lymphoma.[26] Both regimens were effective, but no difference was observed in PFS and OS. Therefore, treatment selection should be based on toxicity associated with treatment, as well as schedule and patient selection. In 2011, the PRIMA study was initiated to assess the potential benefit of 2 years of rituximab maintenance after first-line treatment in patients with high tumour burden FL. The study was designed to show a 45% improvement in median PFS after randomisation when rituximab was given as maintenance in patients who had received R-CHOP, R-CVP, or R-FCM (fludarabine, cyclophosphamide, mitoxantrone) as first-line therapy. The study demonstrated the sustained and persistent benefit rituximab maintenance after immunochemotherapy with a 6-year PFS estimate of 59.2% in the rituximab maintenance arm versus 42.7% in the observation arm.[27,28]

Obinutuzumab, a novel Type 2 humanised anti-CD20 monoclonal antibody engineered for improved antibody-dependent cell-mediated cytotoxicity, was tested for efficacy against rituximab in the 2016 GALLIUM study.[20] Patients with previously untreated, CD20-positive FL, or splenic/nodal/extranodal marginal zone lymphoma were assigned to receive standard chemotherapy with CHOP, CVP, or bendamustine and then randomised (1:1) to rituximab or obinutuzumab.[20] Significantly better outcomes for PFS were reported in the obinutuzumab-based regimen (p<0.001).[20]

Obinutuzumab-based chemotherapy was also assessed in patients receiving bendamustine who were refractory to rituximab (failed rituximab within 6 months of chemotherapy) in the 2016 GADOLIN study.[29] The addition of obinutuzumab significantly improved PFS in these patients (HR: 0.55; 95% CI: 0.40–0.74) and also in patients who were refractory to both alkylators and rituximab (HR: 0.56; 95% CI: 0.40–0.78). Positive data from this trial led to US Food and Drug Administration (FDA) approval of obinutuzumab with bendamustine for the treatment of FL in the relapsed setting. Although these studies are evidence of our incremental progress in FL, most patients are still at risk of relapse in 5–10 years and individual patient responses to therapy still vary greatly. Recent trials have provided valuable insights into the influence of the immune microenvironment in prognosis of patients with FL. OS has been correlated with the molecular features of non-malignant immune cells present in the tumour at diagnosis, underscoring the significance of the tumour microenvironment in clinical outcomes.[6] Such analyses have led to the identification of several key cellular pathways in FL, as well as other lymphomas which are now targetable, including B-cell receptor signalling.[30]

There has been some progress in treating relapsed/refractory patients, who were failing rituximab and were alkylator-refractory, with phosphatidylinositol-3-kinase inhibitors such as idelalisib,[31] duvelisib,[32] and copanlisib.[33] These agents are associated with unique toxicity profiles; idelalisib is associated with nausea and liver function test abnormalities in the first or second cycle. There is also diarrhoea, colitis, and infectious complications. Therefore, it is important to carefully consider the benefits of these agents against their potential side effects in this difficult-to-treat population. Positive data have also been observed with the Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib, hypothesised to improve patient response to rituximab due to its ability to modify the tumour microenvironment. Indeed, high response rates have been observed (82%) when ibrutinib is used in combination with rituximab. AE were primarily Grade 1–2 and in line with the safety profile for ibrutinib.[34] Lenalidomide has also been shown to have excellent synergy with rituximab in patients with untreated, advanced stage indolent NHL.[35] Five-year PFS was reported as 65% (48% for marginal zone lymphoma and 50% in small lymphocytic lymphoma)[34] with similar findings reported in the ALLIANCE study in previously untreated FL patients.[36] In these and other studies in relapsed/refractory FL, the most common Grade 3–4 AE were neutropenia and thrombocytopenia. Lenalidomide plus rituximab is also being investigated for efficacy versus chemotherapy plus rituximab in treatment-naïve patients with histologically confirmed FL Grade 1, 2, or 3a, Stage II–IV in the RELEVANCE trial;[37] versus placebo plus rituximab in patients with relapsed/refractory indolent NHL in the AUGMENT trial,[38] and as maintenance in relapsed/refractory NHL in the MAGNIFY trial.[39]

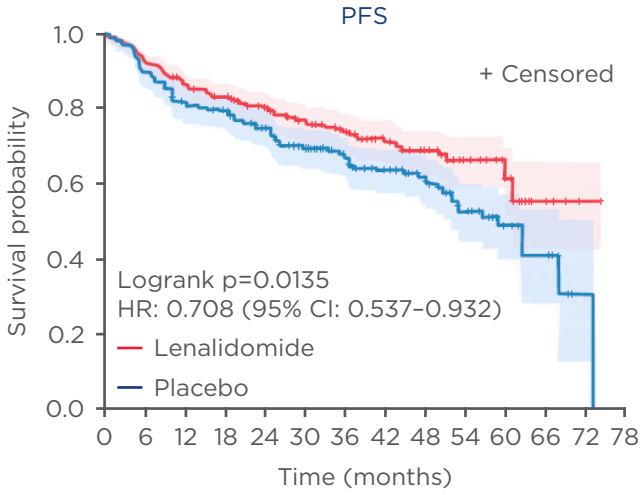
With most current regimens offering similar PFS, treatment should be assessed on an individual basis for patients, bearing in mind the treatment schedule and associated toxicities. Furthermore, considering advances in our understanding of FL biology and development of novel agents with potentially better outcomes, it may be time to look to next-generation agents in an effort to change the natural history of the disease.

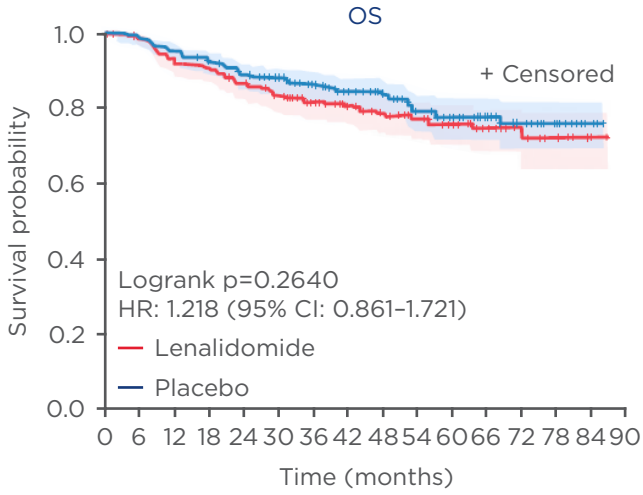
Improving Frontline Treatment Outcomes in Diffuse Large B-cell Lymphoma

Professor Umberto Vitolo

Treatment options available in clinical practice for patients with aggressive lymphoma, specifically for DLBCL, include standard R-CHOP therapy. Sixty percent of patients who have DLBCL respond well to R-CHOP and can be cured of disease,[40] but for the remaining 40% of patients who fail R-CHOP, the chances of salvage are poor.[41] Patients with early relapse and those refractory to prior rituximab treatment have poor response to salvage chemotherapy. Poor outcomes are also observed in young patients undergoing high-dose chemotherapy (despite receiving autologous stem cell transplantation) and in elderly patients with existing comorbidities. Thus, the main goal for DLBCL patients is to improve the efficacy of standard first-line treatment, i.e. R-CHOP. Alternative strategies for these patients may involve: substitution of rituximab with obinutuzumab; intensification of chemotherapy (DA-EPOCH-R); and additional maintenance or inclusion of a novel agent in the existing R-CHOP regimen (X-R-CHOP).

In the GOYA study, 1,418 patients were randomised (1:1) to receive R-CHOP as standard regimen or G-CHOP (CHOP plus obinutuzumab) for six to eight courses with PFS as the primary outcome; however, no significant difference was observed between the treatment arms with regard to PFS or OS.[42] Another approach taken to improve the outcome of DLBCL was to intensify chemotherapy. This was assessed in the Cancer and Leukemia Group B (CALGB)/Alliance Phase III, randomised R-CHOP versus DA-EPOCH-R study in patients with Stage II–IV newly diagnosed DLBCL with event-free survival as the primary endpoint.[43] Again, no significant difference between treatment arms was observed, but there was a significant increase in haematologic toxicity in the dose-adjusted EPOCH-rituximab arm.[43]





No. at risk								
Lenalidomide	323	265	214	137	70	23	1	
	291	250	172	97	42	6	0	
Placebo	327	259	213	137	62	19	1	
	291	250	173	94	42	8	0	

Follow-up: 40 months

No. at risk									
Lenalidomide	323	292	271	217	152	79	27	1	
	312	285	250	188	112	50	12	0	
Placebo	327	308	285	240	164	83	34	3	
	319	299	272	209	117	58	12	0	

Follow-up: 52 months

Figure 2: Lenalidomide maintenance after R-CHOP in elderly DLBCL patients.
CI: confidence interval; DLBCL: diffuse large B-cell lymphoma; HR: hazard ratio; OS: overall survival; PFS: progression-free survival; R-CHOP: cyclophosphamide, adriamycin, vincristine, and prednisone.

In the REMARC study, where elderly patients who responded to R-CHOP were randomised to receive either the immunomodulatory agent, lenalidomide, or placebo as maintenance therapy, an improvement in the primary endpoint, PFS, was reported in the lenalidomide maintenance arm versus placebo (HR: 0.708; 95% CI: 0.537–0.932), but this did not translate into an OS benefit (HR: 1.218; 95% CI: 0.861–1.721) between treatment arms (Figure 2).[44]

Addition of novel agents to standard R-CHOP is the newest strategy under investigation for improving outcomes in the treatment of DLBCL. In a small, non-randomised Phase Ib study, ibrutinib was administered in combination with R-CHOP in patients who had untreated CD20-positive B-cell NHL. Almost all patients responded to treatment, and 70% of them achieved complete remission; however, this was at the expense of a 70% increase in Grade 4 neutropenia.[45] Lenalidomide plus R-CHOP (R2-CHOP) is also currently under investigation, based on its established immunomodulatory profile and its activity in combination with rituximab.[46] Promising results have been reported from two Phase II trials of R2-CHOP in frontline DLBCL, with objective response rates of 98% and 92% and complete response of 80% and 86% reported for the respective studies.[47,48] As evidenced from other studies where additional treatments were added to R-CHOP, there is an observed increase in AE, in particular neutropenia. However, this did not translate into an excess rate of infection or febrile neutropenia, maintaining a similar safety profile to that expected from R-CHOP alone.[47,48]

In addition to exploring new treatment options or combinations, potential strategies to improve patient outcomes include proper identification of poor-prognosis subsets of DLBCL. This includes patients with double-hit lymphoma, who were found to have poor outcomes regardless of induction regimen.[49] Early identification of high-risk patient populations, as defined by concurrent expression of MYC and BCL2 proteins or concurrent presence of *MYC* and *BCL2* translocations, can be critical in guiding therapeutic decisions.[50,51]

Tailoring treatment selection based on genetic or phenotypic characteristics is another option to improve outcomes. Gene expression profiling (GEP) studies in DLBCL have demonstrated that histologically uniform subtypes are biologically distinctive on the molecular level on the basis of their cell of origin (COO). However, because standard GEP tests initially required fresh-frozen biopsy tissues, their use in daily clinical practice was limited. Surrogate immunohistochemistry-based biomarker tests were developed, which, while less sensitive than GEP, could be adopted in a wide range of clinical haematopathology diagnostic laboratories.[52,53] The recent development of a GEP assay for COO classification using formalin-fixed paraffin-embedded tissue sections and a simplified digital gene-expression platform (Lymph2Cx, NanoString Technologies Inc., Seattle, Washington, USA) that can be more easily adopted into clinical practice, is currently under evaluation in a number of clinical trials.

Genetic analyses have revealed two major subtypes: germinal centre B-cell-like (GCB) and activated B-cell-like (ABC), and an additional small subgroup could not be precisely classified into these two entities. Patients with the ABC subtype have been shown to have a lower chance of cure with standard therapy, thus the ability to personalise treatment choices based on molecular subtype can identify patients with a high unmet medical need for non-standard therapy.

When tested in patients treated with R-CHOP, those with the GCB subtype did significantly better than those with ABC.[54] Further studies in these patient subgroups revealed more selective activity of lenalidomide and ibrutinib in the ABC population.[55,56] Ibrutinib was shown to have preferential clinical activity in the ABC subtype in an interim analysis of a Phase II study; ibrutinib produced complete or partial responses in 37% (14/38) of those with ABC DLBCL, but in only 5% (1/20) of subjects with GCB DLBCL (p=0.0106).[56] A hallmark of the ABC subtype is constitutive activation of the NF-κB pathway and as BTK is required for NF-κB signalling in ABC, BTK inhibitors work well in these patients. Lenalidomide was shown to significantly improve PFS over investigator's choice (gemcitabine, rituximab, etoposide, or oxaliplatin) ($p<0.05$), with greater improvements in non-GCB (15.1 versus 7.1 weeks, respectively; p=0.02) compared with GCB (10.1 versus 9.0 weeks, respectively; p=0.55) patients.[57] Similar benefits of lenalidomide on event-free survival were reported when it was added to R-CHOP in ABC patients,[47,58] likely via lenalidomide's immunomodulatory effect on T cell and natural killer cell function in the tumour microenvironment. While R-CHOP remains the standard of care in DLBCL and the backbone of new treatments with novel compounds, ongoing studies such as PHOENIX and ROBUST will provide further insight into the efficacy and safety of ibrutinib and lenalidomide in combination with R-CHOP in the harder-to-treat ABC patient population.[59,60]

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EDITOR'S PICK

Fifteen years ago, renal involvement and renal failure were almost inevitable consequences of multiple myeloma, dramatically impacting survival and quality of life. Since the introduction of innovative drugs (the so called 'new drugs' and 'newer drugs'), autologous haemopoietic cell transplantations, and the innovative technique of light chain removal, myeloma outcomes have clearly improved. Impressive advances have been also achieved in understanding, preventing, and treating myeloma renal complications. This review article reassesses, with extreme clarity, all these findings describing the most frequent manifestations associated with multiple myeloma: myeloma cast neuropathy, AL amyloidosis, and monoclonal immunoglobulins deposition disease, focussing on pathogenesis, mechanism, and therapeutic opportunities.

Dr Emanuele Angelucci

RENAL INVOLVEMENT IN MULTIPLE MYELOMA

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ABSTRACT

Multiple myeloma (MM) is a plasma cell disorder that represents almost 10% of haematologic malignancies. Renal impairment, one of the most common complications of MM that occurs in 20–50% of patients, can present in a variety of forms and is associated with increased mortality. Myeloma cast nephropathy is the most common cause of kidney disease in MM patients, presenting as acute kidney injury in the majority of patients. The recent introduction of new chemotherapy agents, autologous stem cell transplantation, and the development of novel techniques of light chain removal have been associated with improved renal and patient outcomes in MM patients. Nevertheless, dialysis-dependent patients with MM have higher mortality than other dialysis patients and may be considered for kidney transplantation only if sustained remission has been achieved and sustained for at least 3 years, bearing in mind the risk of disease recurrence.

The authors review the most frequent renal manifestations associated with MM, namely myeloma cast nephropathy, light-chain amyloidosis, and monoclonal immunoglobulin deposition disease, focussing on the therapeutic options for acute and chronic kidney disease.

Keywords: Multiple myeloma (MM), renal impairment (RI), pathogenesis, treatment.

INTRODUCTION

Multiple myeloma (MM) is a plasma cell disorder characterised by the presence of >10% of clonal plasma cells in the bone marrow or biopsy-proven plasmocytoma and at least one of the myeloma-defining events (Table 1).[1] MM represents 1% of all malignancies and almost 10% of haematologic malignancies.[2] It is more common in elderly patients with a median age of diagnosis of 70 years, males, and African-Americans.[1,3] Advances in therapy in the last decade have changed the management of this disease and prolonged patient survival.[4,5]

Renal impairment (RI) is one of the most common complications of MM; it occurs in 20–50% of patients and is associated with increased mortality.[6]

Table 1: Diagnostic criteria for multiple myeloma according to the International Myeloma Working Group.

Clonal bone marrow plasma cells ≥10% or biopsy-proven bony or extramedullary plasmacytoma and at least one myeloma defining event	
Evidence of end organ damage: • Hypercalcaemia: serum calcium >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11 mg/dL) • Renal insufficiency: creatinine clearance <40 mL per min or serum creatinine >177 µmol/L (>2 mg/dL) • Anaemia: haemoglobin value of >20 g/L below the lower limit of normal or a haemoglobin value <100 g/L • Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT	**Biomarkers of malignancy:** • Clonal bone marrow plasma cell ≥60% • Involved:uninvolved serum free light chain ratio ≥100 • >1 focal lesions on MRI studies

CT: computed tomography; PET-CT: positron emission tomography–computed tomography; MRI: magnetic resonance imaging.

Table 2: Causes of renal disease in multiple myeloma.

Ig-dependent	Cast nephropathy Monoclonal immunoglobulin deposition disease Light chain amyloidosis Glomerulonephritis: membranoproliferative, diffuse proliferative, cryoglobulinaemic Tubulointerstitial nephritis Fanconi syndrome Minimal change disease Membranous glomerulopathy Immunotactoid/fibrillary glomerulopathy Thrombotic microangiopathy
Ig-independent	Volume depletion Sepsis Hypercalcaemia Tumour lysis syndrome Nephrotoxins (antibiotics, nonsteroidal anti-inflammatory drugs, contrast) Direct parenchymal invasion by plasma cells Pyelonephritis

Ig: immunoglobulin.

It is defined as an elevated serum creatinine (>2 mg/dL) or reduced creatinine clearance (<40 mL/min), as a result of MM.[6] It is important to note the increasing number of cases of RI caused by a monoclonal immunoglobulin (Ig) secreted by a clonal plasma cell disorder that does not meet the criteria for MM, but is consistent with monoclonal gammopathy of undetermined significance. These cases were thus defined as monoclonal gammopathy of renal significance.[7]

Renal disease in MM patients can present in a variety of forms that can be grouped as Ig-dependent, as in the causes that result from the toxic effects of monoclonal light chains, and Ig-independent (Table 2).[8] The toxic effects of monoclonal light chains can affect different segments of the kidney by different mechanisms, which results in a variety of clinicopathologic syndromes, the most common being myeloma cast nephropathy (MCN).[9] Renal biopsy has an important role in the evaluation of MM patients, as different renal lesions have different therapeutic and prognostic implications.[9]

PATHOGENESIS

Monoclonal light chains are freely filtered at the glomerulus and endocytosed and catabolised by the proximal renal tubule cells.[9,10] In MM, the increase

in the levels of monoclonal light chains may exceed the absorptive and catabolic capacity of the proximal renal tubule cells.[9,10] In the distal nephron, the light chains interact with Tamm–Horsfall protein, also named uromodulin, produced by the thick ascending limb of the loop of Henle cells, and form tubular casts, leading to tubular obstruction and inflammation. Cast formation is promoted by hypercalcaemia, dehydration, and nephrotoxins.[9,11]

In the proximal tubule, light chains induce direct toxic effects on proximal tubule cells inhibiting substrate transport, leading to proximal tubulopathy, also named Fanconi syndrome, and promote tubular cell apoptosis and epithelial–mesenchymal transition, leading to tubulointerstitial fibrosis.[11-13]

Glomerulopathy is caused by the deposition of Igs in the amyloid or non-amyloid forms.[9,14] When mesangial cells and macrophages endocytose amyloidogenic light chains and other precursor proteins into lysosomes, amyloid fibril formation takes place.[14] The interaction of fibrogenic monoclonal light chains with mesangial cells induces a fibroblastic phenotype and formation of extracellular matrix.[14,15]

As previously stated, the pathogenesis of MM-associated renal disease also includes Ig-independent mechanisms, which include hypercalcaemia, hyperuricaemia, hypovolaemia, sepsis, rhabdomyolysis, non-steroidal anti-inflammatory drugs, renin–angiotensin system inhibitors, bisphosphonates, and, rarely, pyelonephritis or direct renal parenchymal invasion by plasma cells.[8]

Hypercalcaemia is the second most common cause of RI in MM, impairing renal concentrating ability, causing vasoconstriction of renal vasculature, and enhancing diuresis, resulting in hypovolaemia and acute tubular necrosis.[8] Hypovolaemia, sepsis, and rhabdomyolysis are also frequent causes of acute tubular necrosis in MM patients.[8]

Myeloma Cast Nephropathy and Acute Kidney Injury

MCN is the most common cause of kidney disease in MM patients.[16] Acute kidney injury (AKI) is the most common presentation for MCN, is often associated with dehydration, infection, hypercalcaemia, hyperuricaemia, or nephrotoxins,[17] and in most cases occurs in patients with serum light chains level >100 mg/dL.[16] Proteinuria, primarily light chain proteinuria, is a presenting feature in all MCN patients; however, only 10% have nephrotic syndrome.[17]

Histologically, MCN is characterised by the presence of light chain casts in the distal tubules and collecting ducts, with immunofluorescence staining for a single light chain, and crystalline structure on electron microscopy.[16,17] Also common is a giant cell reaction around the casts, tubular injury, and interstitial inflammation, which may vary from minimal to severe and are dependent on the severity and duration of obstruction.[17] MCN can also coexist with other kidney lesions, such as monoclonal immunoglobulin deposition disease (MIDD) and light chain amyloidosis (AL amyloidosis).[17]

AKI requiring dialysis is seen in ≤10% MM patients, of which 90% have MCN.[18] Hypercalcaemia is the second most frequent cause of AKI in MM patients, and can also predispose to cast formation.[19] Renal insufficiency is associated with higher morbidity and mortality, and is the second most common cause of death in MM patients, after infection, thus emphasising the importance of an early and aggressive treatment,[17,18] because recovery of renal function is associated with increased survival.[20]

Treatment requires elimination of the precipitating factors that favour cast formation, such as correction of hypercalcaemia and dehydration and increasing urine flow, and rapid reduction of serum light chain levels by chemotherapy and extracorporeal removal.[17,18] The benefit of urine alkalinisation is uncertain and might increase the risk of calcification, particularly if hypercalcaemia is present.[16]

The management of myeloma has evolved in the last decade. The current first line of therapy is dexamethasone in combination with bortezomib or with thalidomide, cyclophosphamide, and doxorubicin, followed by autologous stem cell transplantation (ASCT).[3] ASCT has higher response rates and better survival than standard chemotherapy.[17,21] However, the effects of ASCT on renal response and independence of dialysis are variable.[3]

There is only benefit in the extracorporeal removal of light chains when it is associated with effective chemotherapy.[18,22] The largest randomised study in patients with MM and AKI failed to demonstrate benefits of plasma exchange on patient and renal outcomes.[22,23] The current focus is on high cut-off haemodialysis (HCO-HD), which has greater efficacy for light chain removal than

plasma exchange.[24] In fact, the combination of HCO-HD and chemotherapy has been associated with sustained decrease of serum light chains and high incidence of dialysis independence.[25-27] Furthermore, the MYRE trial demonstrated a higher renal recovery rate with intensive HCO-HD when compared with conventional high-flux dialysers, in MM patients with MCN and severe AKI treated with a bortezomib-based regimen.[28] The results of the EuLITE trial will provide further insight on the effects of HCO-HD on patient and renal outcomes.[29]

Chronic Kidney Disease

Chronic kidney disease (CKD) is a common clinical feature of MM. In fact, despite aggressive treatment, progression to CKD Stage 5D occurs in ≤65% of patients with cast nephropathy within 3 months of diagnosis.[17] Patients with MM have a higher mortality rate than other dialysis patients,[17,30] and the survival decreases with more advanced myeloma.[17] In addition, MM treatment-related morbidity and mortality are higher in CKD patients.[31]

Both haemodialysis and peritoneal dialysis are equally effective in patients with MM, with a comparable rate of treatment-related complications.[17,32,33] Kidney transplantation may be considered in patients with MM who achieve sustained remission but remain dialysis-dependent.[8] However, the risks in this population include MM recurrence, infection, and monoclonal Ig-mediated graft dysfunction.[34-37] Therefore, most centres require MM treatment-free remission for at least 3–5 years for a patient to be eligible for kidney transplant.[8,36]

Light Chain Amyloidosis

AL amyloidosis is a systemic disease caused by the extracellular deposition of fibrils.[16] It is most typically associated with lambda light chains.[17] It is the most frequent glomerular lesion in patients with MM, found in ≤30% of cases.[17,38] Approximately 50% have RI at presentation, >70% have proteinuria, and almost 30% present with nephrotic syndrome.[38] Proteinuria is usually mild in patients with vascular limited AL amyloidosis.[38,39] Other systemic symptoms include congestive heart failure, orthostatic hypotension, peripheral neuropathy, diarrhoea, macroglossia, and bleeding diathesis.[38,39]

On light microscopy, amyloid appears as eosinophilic, acellular, and weakly periodic acid-Schiff positive. It is distinguished from other hyaline deposits by their affinity for Congo red dye and birefringence under polarised light.[16,17] After identification of amyloid and further characterisation and typing of the amyloidogenic component, it is essential to determine the target of therapy. On immunofluorescence, the deposits should stain for a single light chain in AL amyloidosis.[38,39] Electron microscopy reveals randomly arranged fibrils with a diameter of 7.5–10 nm.[16,17] The most common kidney compartment affected by amyloid deposits is the vessel walls, but deposits can be found in the mesangium and tubular interstitium.[16]

Progression to CKD Stage 5D occurs in one-third of patients,[39] the median time from diagnosis to initiation of dialysis is ~15 months, and median survival on dialysis ranges from 8–22 months.[16,39] The median survival time in AL amyloidosis patients with nephrotic syndrome is 16 months.[39] Lambda AL amyloidosis, RI, and cardiac involvement are associated with worse prognosis.[17,39] In dialysis AL-amyloid patients, the primary cause of death is cardiac amyloidosis.[16,38,39] Recent therapeutic advances for MM have increased haematologic response and improved median survival.[40-43] Renal response, defined as a 50% decrease in proteinuria in the absence of a 25% increase in serum creatinine or a 25% decrease in glomerular filtration rate, is associated with haematologic response and is a marker of overall survival.[44,45] Due to the poor prognosis of Stage 5D patients with AL amyloidosis, kidney transplant is rarely an option; however, it can be considered in patients without significant cardiac involvement, in combination with successful ASCT.[37]

Monoclonal Immunoglobulin Deposition Disease

MIDD occurs in 25% of MM patients.[17,46] It includes light-chain deposition disease (LCDD), light heavy-chain deposition disease, and heavy-chain deposition disease, the most frequent being LCDD, which accounts for >70% of cases.[16,46,47] In LCDD, kappa light chains are more frequent than lambda light chains.[46,47]

RI and proteinuria are the more common renal manifestations, typically rapidly progressive renal insufficiency and nephrotic range proteinuria.[47,48] Cardiac involvement, such as congestive heart failure, cardiomegaly, or arrhythmias, and liver involvement, namely hepatomegaly, portal hypertension, or hepatic insufficiency, are present in almost 80% of cases.[47,48] Gastrointestinal or neurologic manifestations are present in <30% of patients.[47,48]

On light microscopy, two-thirds of patients present with nodular mesangial sclerosis.[16,17] These nodules are periodic acid-Schiff and silver positive, and Congo red negative. Immunofluorescence stains the specific light, heavy chain, or entire Ig in a linear pattern diffusely along the glomerular and tubular basement membranes and along vessel walls. Electron microscopy reveals electron-dense deposits in the same localisation as in immunofluorescence.[47,48] In ~20% of MIDD cases, there is co-existence of MCN, and less frequently of amyloid deposits.[48]

Patient survival and treatment response appears to be substantially better in LCDD than in systemic AL amyloidosis, with median patient survival reported as high as 14 years in some cohorts.[46,48,49] Renal prognosis is poor, with most patients with MIDD progressing to CKD Stage 5D.[48,49] Age and serum creatinine at presentation are predictors of CKD progression, and age and presence of extra-renal manifestations are also predictors of mortality.[49]

Early diagnosis and treatment is critical and may improve renal outcomes, as clinical remissions of LCDD with resolution of nodular glomerulopathy have been reported.[49,50] Survival of MIDD patients on dialysis is variable, from 7–48 months.[47,48] Due to the poor prognosis of MIDD, kidney transplant is not generally considered, and should be reserved for patients with a complete response to therapy, because the recurrence rate in patients not in complete response is nearly 80%.[35]

Less Common Renal Manifestations

Membranoproliferative glomerulonephritis is a less common MM-related kidney manifestation.[51] It is an immune complex-mediated glomerulonephritis, characterised by subendothelial and mesangial deposition of immune complexes.[51] Most patients present with RI, significant proteinuria, and hypertension.[51] The prognosis is poor, with most patients progressing to CKD Stage 5D, and with frequent recurrence of the disease after kidney transplant.[51]

Fanconi syndrome is a rare disease, characterised by global proximal tubular dysfunction that results from crystalline deposition from a monoclonal light chain, most commonly kappa light chain.[16,52] Most patients present with proteinuria, RI, and electrolyte abnormalities in the serum including hypouricaemia, hypophosphataemia, and hypokalaemia, and urine, including aminoaciduria, glycosuria, and phosphaturia.[52] Rarely, distal tubular dysfunction occurs in association with proximal tubular dysfunction, presenting with distal renal tubular acidosis and nephrogenic diabetes insipidus.[52] The renal prognosis is variable in these patients.[53]

Immunotactoid glomerulonephritis is a rare glomerular disease characterised by the deposition of fibrils in the glomerulus, distinguished from amyloidosis, as its fibrils are larger and do not stain with Congo red dye.[54] It is often associated with a monoclonal gammopathy, and in 12.5% of these cases with MM. The typical presentation is heavy proteinuria, haematuria, and mild RI.[54] Renal failure due to myeloma infiltration has also been documented, but only in 10–31% of autopsy series.[55,56]

CONCLUSION

MM-associated renal failure is associated with significant morbidity and mortality. Therefore, it is important to promptly diagnose and initiate adequate therapy. MCN is the most common cause of renal dysfunction in MM. The recent introduction of new chemotherapy agents and ASCT, and the development of novel techniques of light chain removal, have been associated with improved renal and patient outcomes in MM patients. Nevertheless, dialysis-dependent patients with MM have higher mortality than other dialysis patients, and may be considered for kidney transplantation only if sustained remission has been achieved and sustained for ≥3 years, bearing in mind the risk of disease recurrence.

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TREATMENT OPTIONS IN CARDIOGENIC SHOCK WITH INTRA-AORTIC BALLOON COUNTERPULSATION

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ABSTRACT

Cardiogenic shock (CS), a state of inadequate tissue perfusion due to cardiac dysfunction, remains the leading cause of death following acute myocardial infarction (AMI). While the prognosis of CS post-AMI has improved in recent decades due to advances in treatment modalities, the mortality rates remain unacceptably high (˜40–50% according to recent registries and clinical trials). Current treatment strategies for this condition include early revascularisation to restore blood flow to the ischaemic myocardium, the use of fluids and vasopressor or inotropic agents to reinstate haemodynamic parameters, and initiation of intra-aortic balloon counterpulsation (IABP) systems and active assist devices to maintain circulation. However, there is little evidence that these treatments actually improve survival rates. Even the most recent randomised trial conducted in this field (the IMPRESS trial comparing intra-aortic balloon counterpulsation to the Impella CP mechanical assist device) again failed to demonstrate any improvement in patient outcomes. The lack of evidence may reflect the relatively few randomised trials conducted in this area, likely due to difficulties in conducting such trials in an emergency setting. Moreover, most recent trials have focussed on patients in the late stages of CS, when they have become refractory to medical treatment and require mechanical circulatory support. This article reviews the available literature concerning the treatment of CS post-AMI in light of these limitations, and provides some evidence-based recommendations for best practice, including an updated treatment protocol.

Keywords: Myocardial infarction, cardiogenic shock (CS), microcirculation, intra-aortic balloon pump, mechanical circulatory support (MCS).

INTRODUCTION

Cardiogenic shock (CS) is the leading cause of mortality following acute myocardial infarction (AMI). It is defined as a physiological state of end-organ hypoperfusion due to reduced cardiac output, which can ultimately irreversibly damage vital organs.[1] CS has a wide clinical spectrum, ranging from preshock (hypoperfusion without hypotension, at risk of developing CS) to refractory CS (unresponsive to conventional therapies).[2] Recognising the signs of preshock (e.g. a fall in urine output and rapid heart rate) is critical for early intervention and preventing progression to refractory CS.[2]

Available interventions for CS include fluid boluses (to maintain cerebral perfusion), ventilatory support, revascularisation, pharmacological treatment (e.g. vasopressors and inotropes), and mechanical circulatory support (MCS). Unfortunately, despite available treatment modalities, the mortality rates of CS post-AMI still approach 40–50%;[3] those with refractory CS have the worst prognosis.[2]

Current treatment guidelines for CS post-AMI are mostly based on individual experiences, case series, or registries due to limited evidence from prospectively randomised clinical trials (RCT). Moreover, the RCT conducted so far provide little evidence that, beyond early revascularisation (ERV), the available interventions have any relevant effect on reducing mortality rates in patients with

CS post-AMI. The lack of efficacy may be because RCT have traditionally only altered one element in the recommended treatment protocol at a time, which may not be sufficient to bring about improvements in mortality post-AMI. In addition, most RCT have focussed on the late stages of CS, when patients already require MCS. Considering these limitations, the available literature on the treatment of CS post-AMI was reviewed, with the aim of revising current treatment practices.

CURRENT TREATMENT PARADIGM FOR CARDIOGENIC SHOCK POST-ACUTE MYOCARDIAL INFARCTION

The classic clinical syndrome of CS is diagnosed as hypotension (systemic blood pressure <90 mmHg for 30 min or mean arterial pressure <65 mmHg for 30 min or vasopressors required to achieve blood pressure ≥90 mmHg) with reduced cardiac index (<1.8 L/min/m^2 or <2 L/min/m^2 with support), despite having adequate or elevated left ventricular (LV) filling pressures, with signs of impaired organ perfusion (i.e. altered mental status, cold/clammy skin, oliguria, and/or increased serum lactate).[4] The current treatment strategy for patients with CS post-AMI, based on European and American guidelines and experts' recommendations,[4-7] is summarised in Figure 1. In brief, following diagnosis of CS, coronary angiography is recommended to detect any acute coronary occlusion and restore coronary blood flow.[4-6] Medical treatment with ventilatory support, intravenous fluids, and/or administration of pharmacological agents is then indicated to restore blood pressure and respiration. Subsequently, the three main treatment strategies for restoring organ perfusion and cardiac output in CS post-AMI are revascularisation, MCS (intra-aortic balloon counterpulsation [IABP], ventricular assist devices, and extracorporeal membrane oxygenation [ECMO]), and infusion of vasopressors/inotropes.

Revascularisation in Cardiogenic Shock Post-Acute Myocardial Infarction

Restoration of coronary blood flow (revascularisation) is typically achieved via primary percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG). However, for patients in whom PCI or CABG cannot be performed immediately (within 2 hours), thrombolytic therapy (TT) may be considered.[4,6]

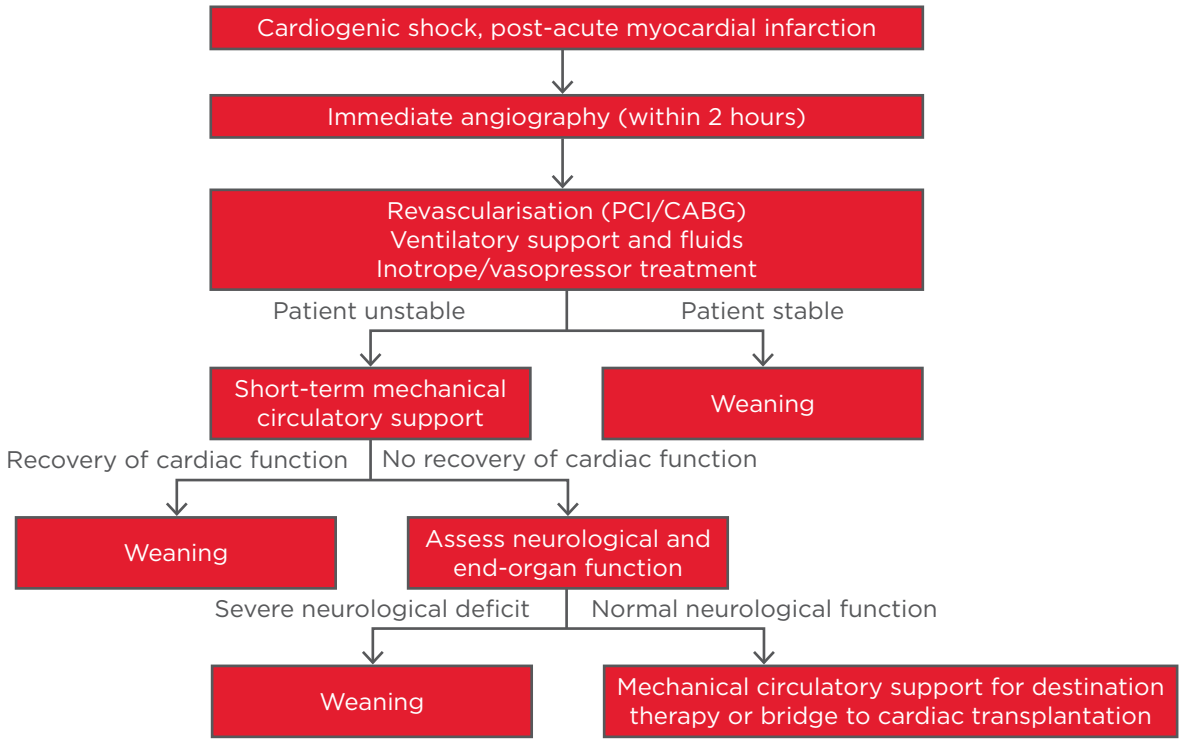

Figure 1: Current treatment strategy for cardiogenic shock post-acute myocardial infarction as outlined in the 2014 European Guidelines.[6]

CABG: coronary artery bypass grafting; PCI: percutaneous coronary intervention.

Adapted from Thiele et al.[7]

Table 1: Summary of randomised clinical trials for treatment of patients with cardiogenic shock after acute myocardial infarction.

Trial name	Clinical trial No.	Trial design	Number of patients	Pharmacological support	Main finding(s)	Reference
N/A	N/A	Single-centre, open-label	IABP: n=20 pLVAD (TandemHeart™): n=21	Heparin, inotropes/ vasopressor, diuretics, and fluids	Haemodynamic and metabolic parameters reversed more effectively with pLVAD than IABP. More complications encountered by pLVAD.	Thiele et al.[22]
TACTICS	N/A	Multicentre	IABP (3 hours after fibrinolysis): n=30* Control: n=27# *25% did not receive IABP #33% of patients crossed over to IABP	Heparin, inotropes/ vasopressor, diuretics, and fluids	IABP use not associated with survival benefit when added to fibrinolysis. NB: Trial was stopped prematurely as it did not achieve the enrolment goal.	Ohman et al.[23]
N/A	N/A	Single-centre, open-label	IABP: n=31 Control: n=9* *27.5% of patients crossed over to IABP	Inotropes/vasopressor, analgesic and anticoagulant agents	Patients treated with inotropes and IABP (in both early and late shock groups) showed a reduction in mortality of 66% and 69%, respectively.	Arias et al.[19]
N/A	N/A	Prospective, multicentre, open-label	IABP: n=14 pLVAD (TandemHeart): n=19	Inotropes, vasopressors, and other pharmacological agents	No difference in 30-day survival or severe adverse events. pLVAD increased cardiac index and mean arterial blood pressure and significantly decreased pulmonary capillary wedge pressure compared to IABP.	Burkhoff et al.[20]
SHOCK	NCT00000552	Multicentre (international)	Early revascularisation (ERV; PCI/CABG): n=152 IMS: n=150	Thrombolytic therapy	No difference in mortality after 30 days (46.7% in the ERV versus 56% in the IMS group). ERV was associated with sustained benefit compared to IMS in the long-term follow-up at 1 year (50.3% versus 63.1%; p=0.027) and at 6 years.	Hochman et al.[8,9]
ISAR-SHOCK	NCT00417378	Prospective, two-centre, open-label	IABP: n=13 pLVAD (Impella LP 2.5): n=13	Heparin, inotropes/ vasopressors, fluids	Higher cardiac index after 30 min in patients treated with Impella than IABP but no difference in 30-day mortality (46% in both groups).	Seyfarth et al.[21]
IABP-SHOCK	NCT00469248	Prospective, single-centre, open-label	IABP: n=19 Control (standard care): n=21	Inotropes/vasopressors, aspirin, glycoprotein-IIb or IIIa receptor-blocker, heparin	IABP use had no significant effect on cardiac index or systemic inflammatory activation.	Prondzinsky et al.[16]
IABP-SHOCK II	NCT00491036	Prospective, multicentre, open-label	IABP: n=300 Control (no IABP): n=298	Inotropes and fluids	IABP did not significantly reduce 30-day mortality.	Thiele et al.[3]
IMPRESS in Severe Shock	NTR3450	Prospective, multicentre, open-label	pLVAD (Impella CP): n=24* IABP: n=24# *One patient already on IABP, one upgrade to Impella 5.0, one received IABP, one received no device #Two patients upgraded to Impella 5.0 and 1 upgraded to pLVAD.	Inotropes/vasopressors in 96% of patients before randomisation	pLVAD not associated with reduced 30-day mortality compared with IABP.	Ouweneel et al.[36]

CABG: coronary artery bypass grafting; ERV: emergency early revascularisation; IMS: initial medical stabilisation; IABP: intraaortic balloon pump; pLVAD: percutaneous left ventricular assist device; PCI: percutaneous coronary intervention.

ERV was flagged as the most important treatment strategy for CS post-AMI in the randomised SHOCK trial (Table 1).[8,9] In the emergency ERV group (n=152), revascularisation (via PCI or CABG) was initiated within 6 hours after randomisation, whereas patients in the initial medical stabilisation (IMS) group (n=150) underwent delayed revascularisation (54 hours post-randomisation on average). While mortality after 30 days did not differ significantly between the two groups (46.7% in the ERV group versus 56% in the IMS group), ERV was associated with sustained benefit compared to IMS at 6 months (50.3% versus 63.1%; p=0.027) and at 6 years.[8,10] A similar benefit of early revascularisation was noted in the SMASH trial.[11]

Although not widely practiced, current guidelines encourage PCI of critical stenosis or unstable lesions in addition to the culprit lesion (Class IIa Level B recommendation in the European guidelines).[6] The effect of culprit lesion-only PCI compared to multi-vessel PCI on mortality in patients with CS post-AMI is currently being explored in the CULPRIT SHOCK trial.[12]

Intra-Aortic Balloon Counterpulsation

Because of its low cost and relatively easy insertion/removal, IABP remains a frequently used MCS device.[13] In patients with CS post-AMI, IABP increases the myocardial oxygen demand ratio (by increasing coronary and systemic circulation via diastolic augmentation) while it lowers the afterload to systolic ejection, and, therefore, increases cardiac output.[14]

Encouraging clinical observations of the benefit of IABP were first demonstrated in a prospective study using the abovementioned SHOCK trial patient registry.[15] In this study, 856 patients with CS were treated with either IABP only (n=279), TT only (n=132), TT and IABP (n=160), or no TT or IABP (n=285). In-hospital mortality among the four groups was significantly different: TT and IABP (47%), IABP only (52%), TT only (63%), no TT or IABP (77%). The authors concluded that IABP combined with revascularisation (PCI/CABG) lowered in-hospital mortality rates compared with standard medical therapy; however, in cases where early revascularisation is not possible, TT and IABP should be initiated, followed by immediate transfer to a hospital with PCI/CABG capabilities. This study was somewhat limited by selection bias, as patients selected for IABP/TT were a lower-risk group who may have been preselected for a more favourable outcome (e.g. younger age, fewer comorbidities). Therefore, a RCT was warranted to confirm the benefits of IABP.

The first RCT to examine whether IABP in addition to PCI ameliorates multi-organ dysfunction syndrome in patients with CS post-AMI was the IABP-SHOCK trial (Table 1).[16] Among the 45 patients included, no significant difference was observed between those receiving IABP and those receiving standard care with respect to severity of illness, cardiac index, or systemic inflammatory activation.[16] Moreover, additional IABP treatment did not result in significant haemodynamic improvement compared with medical therapy alone.[17] However, there were some limitations of this study, including discrepancies among patients within the groups.[16]

Following the IABP-SHOCK trial, a systematic review and meta-analysis of the literature published up to 2011 confirmed that IABP did not improve survival.[18] However, the results of this meta-analysis were limited by several issues including the small number of available trials (only seven eligible studies[3,16,19-23]), insufficient sample sizes, and heterogeneity of included patients at baseline.[18] There was also frequent crossover in several trials, and different durations of IABP support were used (ranging from 26±19–84±54 hours).[16,18-20] These limitations made it difficult for any definitive conclusions to be drawn. The IABP SHOCK II trial[3] (Table 1) was the first large prospective, randomised, open-label, multicentre trial, to investigate the influence of IABP support on mortality. Following presentation, patients with CS were randomly assigned to either IABP (n=300) or conventional treatment (n=298). The SHOCK II trial proved IABP is a safe therapy and offers haemodynamic benefits in CS.[3] However, in terms of the primary endpoint of 30-day mortality, there was no significant difference between the two treatment groups (39.7% for IABP versus 41.3% for control; p=0.69). The 12-month follow-up data confirmed there was no significant difference in mortality rates between the two treatment groups (52% for IABP versus 51% for control; p=0.91).[24] Moreover, in the survivors, quality-of-life measures (mobility, pain, depression, and others) were similar between the two treatment groups.[24]

The negative results of the SHOCK II trial were somewhat controversial, given IABP has been used in CS for several decades. However, the lack of benefit may reflect several limitations of the study. For example, 10% of the conventional treatment

(no IABP) group crossed over into the IABP group and some patients in the control group received another MCS device.[25] In addition, the timing of the IABP insertion was not standardised and was left to the discretion of the operator (IABP was inserted pre-PCI in 13.4% of cases).[3] Indeed, IABP insertion after PCI was previously shown to be associated with increased in-hospital mortality.[26] Furthermore, the trial was not sufficiently powered to assess a difference in mortality: given the observed mortality rate of ˜40%, >900 patients would have been required to detect the effect compared to the 600 enrolled.[27] Nevertheless, as a consequence of the SHOCK II trial, IABP was downgraded to a Class IIa Level B recommendation, Level of Evidence B (can be considered in patients who do not quickly stabilise with pharmacological therapy) in the current American guidelines.[5] Furthermore, IABP is not routinely recommended in the latest European guidelines (Class III recommendation, Level of Evidence B) but may be used as an adjunct for patients with mechanical complications as a bridge to surgery.[28]

A recent advance in IABP has been the use of a larger capacity 50 cc IAB, which showed greater systolic unloading and diastolic augmentation compared to standard 40 cc IABP in a real-world clinical study.[13] The use of the large-volume 50 cc IABP as a first-line MCS strategy (with escalation when needed) was retrospectively analysed in a single-centre observational study of 100 patients with CS, with a promising rate of overall survival to hospital discharge of 66%.[29] However, a RCT is needed to further validate these findings.

Percutaneous Left Ventricular Assist Devices

Percutaneous left ventricular assist devices (pLVAD) represent a newer type of MCS, and are used as a bridge to recovery or transplant, or as permanent (destination) therapy. Currently available pLVADs include TandemHeart™ (Cardiac Assist, Pittsburgh, Pennsylvania, USA); Impella® 2.5, Impella® 5.0 (needs to be surgically implanted), and Impella® CP systems (Abiomed Europe, Aachen, Germany); and the paracorporeal pulsatile device iVAC 2L® (PulseCath BV, Arnhem, Netherlands).[30] TandemHeart and Impella have shown improved short-term haemodynamic support; however, trials have yet to be conducted using iVAC2L.

Thiele et al.[22] were among the first to report that patients with CS post-AMI who were randomised to receive pLVAD with TandemHeart (n=21) more rapidly showed improved haemodynamic and metabolic parameters than those receiving standard treatment with IABP (n=20; Table 1).[22] However, pLVAD resulted in more complications, including bleeding/vascular complications.[22,31-33] With regard to Impella, Seyfarth et al.[21] reported it was safe to use and provided superior haemodynamic support at 30 min, but showed similar haemodynamic results to IABP at all other time points in a RCT involving 26 patients with CS (ISAR-SHOCK,[21] Table 1). Although the study by Seyfarth et al.[21] showed a higher cardiac index in patients treated with Impella compared to IABP, the overall mortality was similar (46%) in both groups.[21] However, the small RCT by Thiele et al.[22] and Seyfarth et al.[21] lacked enough power to show any differences in clinical outcomes.[18]

A subsequent meta-analysis indicated that although pLVAD may provide superior haemodynamic support in CS compared to IABP, it does not improve early survival.[34] As further evidence, a registry comparing Impella 2.5 to IABP in the setting of post-resuscitation shock reported similar mortality: the mortality rate was 77% among 35 patients treated with Impella and 79% in 43 patients treated with IABP.[35] More recently, a single-centre RCT (IMPRESS, Table 1) compared IABP (n=24) and Impella CP (n=24) in patients with severe CS post-AMI, and found Impella CP did not reduce 30-day mortality compared to IABP.[36] Mortality was 50% in patients treated with IABP and 46% in those treated with pLVAD (hazard ratio [HR] with pLVAD: 0.96; 95% confidence interval [CI]: 0.42–2.18; p=0.92).[36] Moreover, at 6 months, mortality rates for both Impella CP and IABP were 50% (HR: 1.04; 95% CI: 0.47–2.32; p=0.923).[36] Bleeding occurred more often in Impella-treated patients than in IABP-treated patients. The authors concluded pLVAD shows no additional benefit over IABP in CS post-AMI.[36] However, it must be noted that the patients included in this study were in the end-stage of disease when they required mechanical support. Additionally, this study had a small sample size and was underpowered to show clinical outcome. In addition, some crossovers and upgrades occurred in this trial, at the discretion of the investigator, which may have affected the results. Moreover, the IMPRESS trial did not compare Impella to standard treatment, which is now being investigated in a Danish multicentre trial (DanShock).[37]

Currently, Impella is recommended in patients with severe LV dysfunction, and is approved by the US Food and Drug Administration (FDA) to provide

short-term circulatory support for ≤6 hours; in Europe, it may be used for ≤5 days.[38] Abiomed's instruction for the use of Impella 2.5 L and the CP device for CS is ≤4 days and during high-risk PCI it is <6 hours.[39] In addition to pLVAD, the use of ECMO should be considered for concomitant hypoxaemia.

Extracorporeal Membrane Oxygenation

ECMO, also known as extracorporeal life support, is a reliable method for urgent resuscitation in severe refractory CS and during transportation.[40] While ECMO decompresses the venous system, provides flow, and ensures oxygenation, it cannot unload the left ventricle like pLVADs.[41] Therefore, ECMO alone may cause progressive distention, and, subsequently, further LV failure. To address this, a recent case report showed the combined use of ECMO and Impella 5.0 could effectively manage CS post-AMI to prevent multi-organ damage as a bridge to transplantation.[42] However, to date, no large RCT has been conducted on the use of ECMO in CS post-AMI.

Management of Haemodynamic Parameters with Vasopressor and Inotropic Agents

Irrespective of early revascularisation by PCI/CABG with or without MCS, volume expansion with vasopressors and/or inotropes remains a cornerstone of haemodynamic support in CS.[4,7] However, excessive increases in volume and systemic vascular resistance may increase cardiac afterload and worsen cardiac failure. Indeed, according to a recent Cochrane review, neither inotropic nor vasopressor therapy reduced mortality rates in CS post-AMI.[43] Instead, these agents were associated with an increase in clinically relevant arrhythmia. Inotropic and/or vasopressor therapy may also induce myocardial ischaemia, and, in some cases, even result in hypotension.[44] Moreover, inotrope use has been shown to increase the mortality risk by ≤80%.[45] Therefore, it appears that these drugs worsen the ischaemia-related imbalance of oxygen supply and demand in AMI.

When considering the type of vasopressor/inotrope to use, dopamine was previously recommended for use as a first-line vasopressor agent for CS post-AMI; however, RCT evidence suggests that norepinephrine is safer in this setting.[46] Meanwhile, epinephrine was associated with increased 90-day mortality and worsening of cardiac and renal injury in the multinational CardShock study, which analysed the use of vasopressors and inotropes in 219 patients with CS.[47] This study also found levosimendan combined with norepinephrine, as well as dobutamine combined with norepinephrine, had better outcomes, and may be a preferable treatment for CS.[47] Nevertheless, more RCT are required to confirm these results, such as the OptimaCC study.[48]

Another trial is currently evaluating the efficacy and safety of intravenous epinephrine infusion as an early and fast haemodynamic stabiliser, associated with tight tissue perfusion monitoring, in the context of a stepwise progression in the treatment of CS, including MCS.[49] At present, however, there is no clear evidence for recommendation of the use of vasopressors/inotropes in CS, although it is advised that if they are implemented, the lowest dose possible should be used and patients should be weaned as soon as possible.[5,6]

RETHINKING THE TREATMENT STRATEGY FOR PATIENTS WITH ACUTE MYOCARDIAL INFARCTION COMPLICATED BY CARDIOGENIC SHOCK

As vasopressors and inotropes may worsen cardiac and renal injury, their use may be negating the benefits of MCS-related trials. As evidence, a recent study demonstrated that norepinephrine had a negative effect on the response to IABP-related microflow improvement in CS.[50] So, should the use of vasopressors/inotropes in the treatment of CS post-AMI be reconsidered? And if so, what would be the best alternative?

A recent multivariate analysis showed implementation of MCS before PCI (p=0.04) and before requiring inotropes/vasopressors (p=0.05) was associated with increased survival.[51] Survival was 66% when MCS was initiated <1.25 hours from shock onset, 37% when initiated within 1.25–4.25 hours, and 26% when initiated after 4.25 hours (p=0.017).[51] In addition, survival was 68%, 46%, 35%, 35%, and 26% for patients requiring 0, 1, 2, 3, and ≥4 inotropes before MCS support, respectively.[51] So early MCS prior to PCI or vasopressor/inotrope therapy seems to be an attractive option, but does IABP or pLVAD provide the best outcome?

Some reports have demonstrated improved survival in patients who received IABP before primary PCI compared to post-PCI (i.e. mortality rates of 25% versus 55%, respectively).[26]

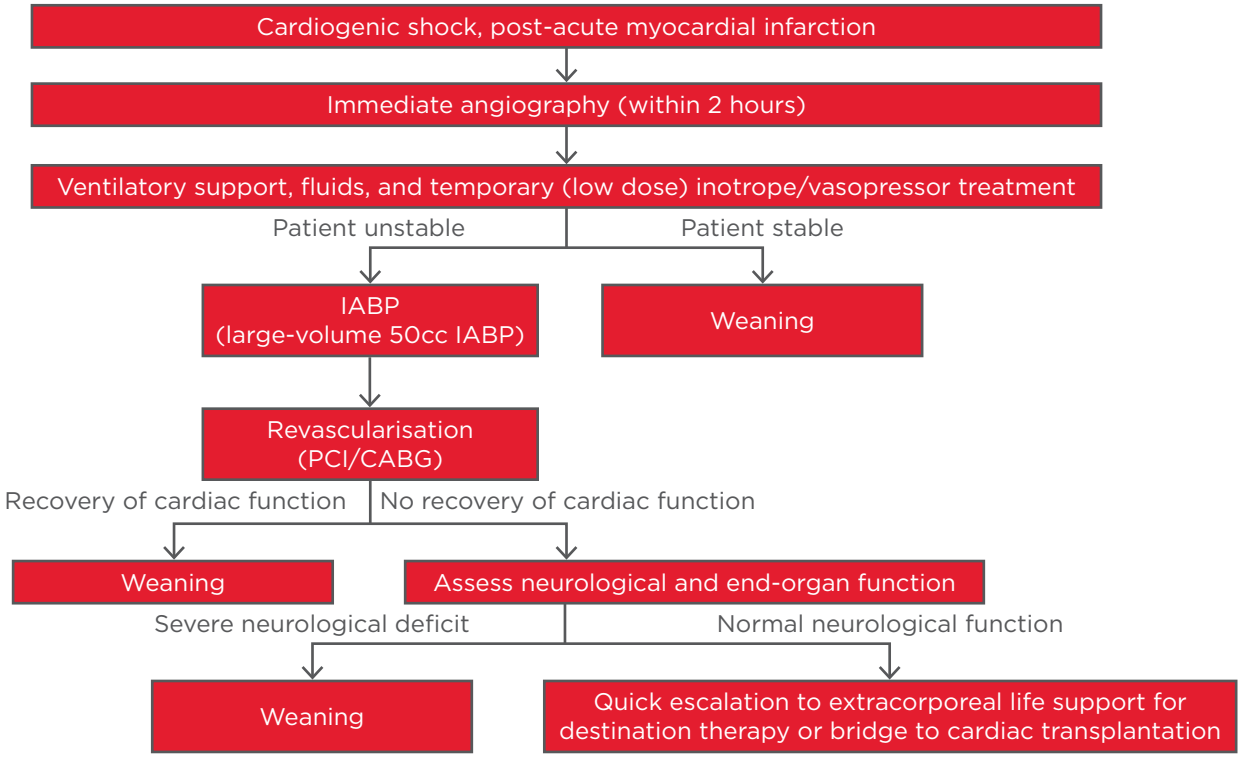

Figure 2: Proposed new treatment strategy for cardiogenic shock post-acute myocardial infarction based on currently available literature.

CABG: coronary artery bypass grafting; IABP: intra-aortic balloon pump; PCI: percutaneous coronary intervention.

Indeed, as IABP only provides minor circulatory support and requires a certain level of residual LV function, implementing it too late in the treatment timeline may be useless. Clinical data from the USpella registry also indicate that mortality is reduced in patients supported by pLVAD (Impella) prior to PCI.[52] However, current RCT indicate pLVAD is associated with increased vascular/bleeding complications,[22,31-33] and has higher associated costs compared to IABP.[53]

While many standardised treatment protocols for managing CS post-AMI have been established, the use of these tools still varies across centres. Based on current evidence, a new treatment algorithm is proposed (summarised in Figure 2). From this algorithm, a more uniform patient management strategy could potentially be developed for implementation in multiple centres worldwide.

In the treatment strategy proposed here, IABP (specifically using the higher efficacy 50 cc IAB) is now recommended as the first-line therapy and should be initiated prior to PCI, although this recommendation would require additional guidelines to be developed for early identification of patients for whom IABP is likely to be sufficient. Another recommendation is that low dose (at most) inotropes/vasopressors should be used only as temporary measures until IABP can be initiated prior to PCI; this may improve survival rates while avoiding the mortality associated with inotropes and vasopressor agents.[54] Finally, if patients fail to stabilise, it is recommended that a quick escalation to full support using ECMO should occur. However, adequately powered RCT are required to validate the new treatment strategy.

CONCLUSIONS AND THOUGHTS FOR THE FUTURE

CS post-AMI remains challenging to treat and high mortality rates warrant further RCT in this setting. To do this, we must first overcome the inherent difficulties in conducting such trials in the emergency setting of AMI complicated by CS, as outlined previously.[55] Future studies should also investigate using the available interventions at earlier stages of the disease pathology. Nevertheless, the available literature strongly indicates that it is time to reassess the standard treatment protocols for AMI patients suffering CS.

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PEYRONIE'S DISEASE: RECENT TREATMENT MODALITIES

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ABSTRACT

Peyronie's disease (PD), which is characterised by fibrous plaque formation in the tunica albuginea of the penis, is associated with pain, erectile dysfunction, and anatomical malformations that negatively affect the quality of life of afflicted men. The optimum medical therapy for PD has not yet been identified. In the last 5 years, commonly used oral medications have been replaced by intralesional therapies. Intralesional collagenase *Clostridium histolyticum* is the only US Food and Drug Administration (FDA) approved treatment for PD. Minimally invasive intralesional therapies and surgical intervention form the basis of contemporary therapy for this disorder. These therapeutic options, along with selected portions of the guidelines, are explored in this review. The objective is to describe the current state of practice for each of the most commonly used, as well as several developing, treatment modalities of PD.

Keywords: Peyronie's disease (PD), treatment, surgery.

INTRODUCTION

Peyronie's disease (PD) is a connective tissue disorder of the penis that is psychologically devastating for affected men and leads to penile deformity. This wound healing disorder is thought to result from trauma or microtrauma to the erect penis in genetically susceptible individuals, though the mechanism of disease has not yet been fully explained.[1] Disease prevalence is often quoted at 3.2–8.9% in adult men, though it may be higher due to under-reporting. The most commonly associated comorbidities and risk factors are diabetes, hypertension, lipid abnormalities, ischaemic cardiopathy, erectile dysfunction (ED), low testosterone, smoking, and excessive consumption of alcohol.[2-4] Dupuytren's contracture is more common in patients with PD and affects 9–39% of patients.[5-7]

The pathophysiological basis for PD relates to the inflammation seen in the acute phase (painful erections, 'soft' nodule/plaque) and the subsequent disordered wound-healing, which is characteristic of the chronic phase (disease stabilisation).

The diagnosis of PD involves a focussed history considering the presenting symptoms and erectile function status. Physical examination must include assessment of penile length, palpable nodules, and extent of curvature.[8] The assessment of penile curvature occurs during an erection. This can be obtained by a home (self) photograph of a natural erection, which is preferable, or by means of a vacuum-assisted erection test or intracavernosal injection using vasoactive agents.[9] Ultrasound measurement of the plaque's size is inaccurate, and it is not recommended in everyday clinical practice.[10] Doppler ultrasound may be required for the assessment of vascular parameters.[11]

Once the diagnosis is made, the patient should be counselled on the available treatment options. The presence of ED and the related psychological factors may impact treatment strategy.

DISEASE MANAGEMENT OF PEYRONIE'S DISEASE

Non-surgical Treatment

Conservative treatment of PD is primarily focussed on patients in the early stage of the disease (Table 1).

Table 1: Non-surgical treatment options.

Oral treatments	Intralesional treatments	Other treatments
• Vitamin E • Potassium para-aminobenzoate • Tamoxifen • Colchicine • Acetyl esters carnitine • PTX • PDE5i	• Steroids • Verapamil • Clostridium collagenase • Interferon	• Stem cells • Lontophoresis • ESWT • Traction devices • Vacuum devices

ESWT: extracorporeal shock wave treatment, PDE5i: phosphodiesterase Type 5 inhibitor, PTX: pentoxifylline.

Oral Treatment

Vitamin E

Vitamin E has been extensively investigated for its potential use in the treatment of PD. According to guidelines and earlier research, vitamin E is not currently recommended for the treatment of PD.[8]

Tamoxifen citrate

Tamoxifen citrate is a non-steroidal oestrogen receptor antagonist used in the treatment of breast cancer. This medication has been explored as a therapeutic option for PD due to its inhibitory effects both on the release of transforming growth factor (TGF) from fibroblasts and TGF-receptors.[12] As a treatment for PD, tamoxifen is not recommended in the guidelines.[8]

Potassium para-aminobenzoate

Potassium para-aminobenzoate is an anti-fibrotic agent with monoamine oxidase activity that is used to treat fibrotic conditions, such as Dupuytren's contracture. Overall, para-aminobenzoate use in PD should be approached with caution given its questionable efficacy and its potentially severe side effects.[13]

Colchicine

Colchicine is known as a treatment for gout and its proposed mechanism of action in PD is on the basis of its anti-inflammatory effect.[14] This medication as a treatment for PD has been abandoned.

Carnitine

Propionyl-L-carnitine is a short-chain acyl derivative of carnitine and acts as an acetyl-coenzyme A inhibitor. Its role in the treatment of PD stems from its anti-oxidant properties, previously utilised for idiopathic infertility, and its antiproliferative effects on endothelial cells.[15] Carnitine is currently not recommended in the guidelines for PD.[8]

Pentoxifylline

Pentoxifylline is a non-specific phosphodiesterase inhibitor with anti-inflammatory and antifibrogenic properties. Pentoxifylline is not recommended in the guidelines for the treatment of PD.[8]

Non-steroidal anti-inflammatory medications

The clinician may offer oral non-steroidal anti-inflammatory medications to patients suffering from active PD who are in need of pain management.[8]

Phosphodiesterase Type 5 inhibitors

Phosphodiesterase Type 5 inhibitors (PDE5i) have long been used in the treatment of ED. In PD, these agents are proposed to inhibit tissue remodelling after acute injuries by decreasing oxidative stress responsible for inflammation and fibrosis.[16] Therefore, no recommendation can be given for PDE5i in patients with PD.

Topical H-100 gel

H-100 gel is composed of nicardipine, superoxide dismutase, and emu oil. One study demonstrated a significant improvement in flaccid–stretched penile length, curvature, and pain level in 22 patients, thereby opening the door for future research into the suitablility of H-100 gel as a treatment for acute phase PD. H-100 gel is a safe and possibly effective non-invasive, topically applied treatment. A self-limited rash was the only side effect in three patients. However, more safety and efficacy data from larger trials are needed prior to routine usage.[17]

Stem cell treatment

PD is often associated with antecedent trauma to the erect penis. It is some interplay between

trauma and genetic susceptibility that leads to the development of the disease. This idea is supported by the presence of myofibroblasts in PD lesions. These myofibroblasts originate from pluripotent stem cells in the tunica albuginea, but, importantly, they are not present in normal tunica albuginea tissue.[18]

The therapeutic action of these stem cells is thought to be derived from their proangiogenic capacity that alters the cycle of vascular injury, ischaemia, and fibrosis characteristic of the inflammatory phase of PD.[19]

Castiglione's group has been using stem cells to assess improvement of both PD and ED in rat models. Despite the lack of any long-term results, they showed that they could induce PD and ED with TGF-β injections. When they used human adipose tissue-derived stem cells, they noted a reduction in fibrosis and improvement in erectile function.[20] Later, Levy's group published a prospective trial of stem cells in five patients suffering from PD. In this study, 7 of the 10 plaques initially seen with ultrasonography disappeared completely at 3-month follow-up. The results seemed promising.[21]

While still in its infancy, stem cell therapy offers perhaps the best hope for a definitive PD cure.

Intralesional Therapies

Intralesional injection therapy has been used for years to treat PD. Adequate drug penetration may significantly slow, prevent, or reverse PD plaque formation. Higher concentrations injected immediately into cells should hopefully negate the need for prolonged treatment as seen with some oral medications. Unfortunately, results have been limited and many medications are riddled with local side effects including pain, bruising, and local inflammation.

Intralesional collagenase

Collagenase Clostridium histolyticum (CCh) is an enzyme that degrades interstitial collagen, making it a logical choice in the treatment of PD. It is approved for the treatment of chronic dermal ulcers and severe burns. It has also been successfully used in the treatment of Dupuytren's contractures, with which PD shares a similar pathophysiology.[22]

CCh is now approved by the US Food and Drug Administration (FDA) for PD in adult men with a palpable plaque and a curvature deformity of ≥30° at the start of therapy. Intralesional CCh is a purified mix of two collagenases that leads to a breakdown of the collagen when injected into the PD plaque, which can lead to a reduction in penile curvature.[23-25]

The outcomes of IMPRESS I and II have been published. There were significant results at 1-year follow-up: a mean improvement of 17° in penile curvature at 36 weeks and an improvement in erectile function.[26,27]

Intralesional treatment with CCh showed significant decreases in the deviation angle, plaque width, and plaque length. CCh is contraindicated for atypical PD patients including ventral plaques and/or 'hourglass' deformities.[22,28] CCh is associated with minor local side events including penile ecchymosis, swelling, and pain. Serious side effects occurred in approximately 1% of intralesional CCh-treated patients. Corporal rupture (penile fracture) requiring surgical repair was reported as an adverse reaction in 5 of 1,044 (0.5%) patients.[29] A combination of penile ecchymosis or haematoma, sudden penile detumescence, and/or a penile 'popping' sound or sensation were reported; in these cases, a diagnosis of corporal rupture could not be excluded. It has been recommended that patients should avoid intercourse for ≥2 weeks after an intralesional CCh injection.[26,27] Because of these side effects, clinicians should inform patients with PD prior to beginning treatment with intralesional CCh regarding the potential occurrence of known side effects.[26,27] Intralesional CCh remains the best-studied intervention for PD and is currently the only pharmaceutical intervention that has been FDA-approved.

Intralesional interferon-α2b

Interferons are endogenously produced cytokines that are responsible for regulating the immune response to antigenic insults.[30] Interferon-alpha2b (IFN-α2b) is thought to improve curvature and reduce plaque size in PD by decreasing the rates of fibroblast proliferation and collagen synthesis.[31] Recent studies have suggested that IFN-α2b leads to an improvement in penile haemodynamics, supporting improved erectile function.[32]

IFN-α2b is a reasonable alternative to CCh as an intralesional treatment, with modest efficacy and an overall excellent safety profile. Intralesional IFN-α2b for ventral penile plaque has similar outcomes and no increased rate of complications compared to dorsal plaques.[33]

The side effects include myalgias, arthralgia, sinusitis, fever, and flu-like symptoms. They can be effectively treated with non-steroidal anti-inflammatory drugs before interferon injection.[34]

Further studies are needed to better compare its safety and efficacy compared to other treatments and to assess its functional significance for patients.

Intralesional verapamil

In vitro calcium channel blockers (CCBs), such as verapamil, have been shown to increase collagenase activity and decrease fibroblast proliferation.[30] Reports on the efficacy of intralesional verapamil in PD are varying. In particular, intralesional verapamil improved penile curvature and subjective PD symptoms, particularly in younger patients, without causing any major complications.[35]

Overall, these findings suggested that intralesional verapamil injections could be advocated for the treatment of non-calcified acute phase or chronic plaques to stabilise disease progression or possibly reduce penile deformity, although large scale, placebo-controlled trials have not yet been conducted.[36]

Intralesional corticosteroids

Intralesional corticosteroids were first used for the treatment of PD in the 1950s.[1] As unfavourable side effects, including local tissue atrophy and fibrosis, made any subsequent surgical interventions more difficult,[30] steroid injections are no longer recommended for the treatment of PD.[8]

Intralesional hyaluronic acid

Hyaluronic acid (HA) is a glycosaminoglycan that has been shown to regulate the immune system by decreasing inflammatory cytokines and thus has been used in multiple medical fields to reduce inflammation and scar formation.[37] HA is a promising novel therapy for PD that appears to have some efficacy in improving PD symptoms, but data comparing HA treatment to placebo or alternative therapies are lacking.[38] Further prospective randomised clinical trials (RCTs) will need to be performed prior to the routine recommendation of HA.

Intralesional botulinum toxin

Botulinum toxin is used in a number of medical fields to reduce fibrosis and scarring. With this in mind, one study evaluated botulinum toxin type A as a treatment for PD.[39] However, more safety and efficacy data from larger trials are required prior to routine usage.

Topical Treatment

Although a topical treatment approach to PD is appealing to patients for reasons of comfort and accessibility, in practice the results are less than ideal.

Topical verapamil

There is no evidence that topical treatments applied to the penile shaft result in adequate levels of the active compound within the tunica albuginea. However, in the largest RCT to date, there was statistically significant improvement in curvature and plaque size in patients receiving topical applications of verapamil (15% twice daily) over the course of 3 months.[40] The effect was statistically superior to that of a placebo, and patients who continued the topical therapy after the 3-month trial continued to show improvement throughout the 9 months they were followed. Further studies are needed to reconcile these contradictory findings.

Iontophoresis

Iontophoresis, or transdermal electromotive drug administration, is a method theorised to provide superior tissue penetration for the transdermal application of medications. An unblinded RCT of 60 patients suggested that verapamil administered with iontophoresis had better results than verapamil administered intralesionally.[41] Again, further large-scale studies are needed to validate these findings.

Non-Pharmacological Treatment

Penile traction devices

Penile traction devices (PTDs) have been studied as a treatment for straightening the penile curvature in men with PD.[42] Some studies have shown ≤25° reduction in curvature, an improvement in sexual function, and a significantly lower risk of surgical intervention.[43] It is likely that PTDs will play a more important role in the future as part of combination therapy for early-stage PD. There are no serious adverse events, including skin changes, ulcerations, hypoesthesia, or diminished rigidity. Further studies are needed to define the role of PTDs in treating PD.

Vacuum devices

The application of vacuum devices follows the same principles as traction devices with the drawback

of being non-continuous and therapy precluding remodelling of the plaque.

Extracorporeal shock wave therapy

Extracorporeal shockwave therapy (ESWT) has been utilised as a treatment for PD, particularly with the goal of reducing pain.[44] Overall, the researchers found ESWT effective for significant pain but not for deviation, plaque size, or sexual function.[45] ESWT cannot be recommended as a treatment for PD, according to German researchers.[46]

Radiotherapy

According to the American Urological Association (AUA), given the potential risks of exposing patients to radiotherapy (RT) in the context of unproven benefits, the panel interpreted these data to mean that RT should not be offered to patients with PD.[8]

Surgical Treatment

Surgical management of PD is indicated for patients with a deformity that impairs sexual function, such as severe penile curvature, penile instability due to an 'hourglass' deformity, or other narrowing deformities. Patients who have failed minimally invasive therapy, have underlying refractory ED, have stable disease, or who desire rapid and reliable results may be surgical candidates as well.[47] Surgery is indicated when PD is stable for ≥3 months (without pain or deformity deterioration), which is usually the case after 12 months from the onset of symptoms, and intercourse is compromised due to deformity. The goal of surgical treatment is to straighten the penile curvature deformity, preserve or restore erectile function, and preserve penile length and girth.[48,49]

Penile shortening is present in almost all patients with PD. Thus, it is important to document the penile length prior to surgical intervention so patients realise that the length loss postoperatively is mainly the result of the PD and not the surgery.[50]

Plication

Penile plication is an option for patients with penile curvature ≤60° and adequate erectile function, either with or without the use of erectile aids. Operative techniques for penile plication surgery have evolved over time. However, a lot of different modifications have been described and the level of evidence is not sufficient to recommend one method over another.[51]

Penile plication is the most commonly offered surgical intervention to patients with PD, and has a success rate of 82–90% for the resolution of penile curvature.[52] The most common complications of surgery are pain and penile haematoma or swelling. Importantly, the final length of the penis following this procedure is equivalent to the length of the shorter side of the penis prior to plication.

Plaque incision or excision with grafting

Graft incision and grafting is indicated for the patient with a complex penile curvature deformity >60°, a large size plaque, a destabilised 'hourglass' or hinge configuration, or a short penile length who can achieve adequate rigidity with or without pharmacologics.[47,53] A number of grafts have been used, including autologous (derived from the individual's own body), allografts (derived from a donor of the same species), and xenografts (derived from different species) (Table 2). More recently, the use of buccal mucosa grafts has been advocated. Buccal mucosa grafts provided excellent short-term results, suggested by the fast return of spontaneous erections and prevented shrinkage, which is the main cause of graft failure. It also proved to be safe and reproducible, thus representing a valuable treatment option for PD.[54] Due to concerns of the risk of infection and fibrosis, synthetic grafts are not recommended.[48] Success and satisfaction rates vary with the different procedures. A RCT evaluating the surgical outcomes between 61 patients undergoing penile plication and 81 patients undergoing plaque excision with grafting found outcomes of penile rigidity, penile length, penile sensation, and the ability to achieve orgasm to be comparable between the two groups.[55] Overall, 82% of plication patients and 75% of plaque excision with grafting patients reported being very satisfied or satisfied postoperatively.[55]

Most recently, Hatzichristodoulou et al.[56] described the feasibility and efficacy of collagen fleece as a promising new graft material. Grafting was performed by a ready-to-use collagen fleece coated with tissue sealant, following partial plaque excision/incision.[56] In that study, total straightness was achieved in 83.6% of patients. Three patients required surgical drainage because of subcutaneous haematoma formation. Glans sensation was normal in 56 patients (91.8%). Major advantages are decreased operative times and easy application. However, long-term clinical outcomes are necessary to confirm these encouraging findings.

Table 2: Types of grafts used in Peyronie's disease surgery.

Autologous grafts	Allografts	Xenografts	Synthetic grafts
• Dermis • Vein grafts • Tunica albuginea • Tunica vaginalis • Temporalis fascia • Buccal mucosa	• Cadaveric pericardium • Cadaveric fascia lata • Cadaveric dura matter • Cadaveric dermis	• Porcine small intestinal submucosa • Bovine pericardium • Porcine dermis	• Gore-Tex® • Dacron

The ideal graft has not yet been found. Surgical experience, along with erectile function, penile length, degree of curvature, and patient preference, all remain important variables in terms of graft selection.[57]

Penile prosthesis implantation

Placement of an inflatable penile prosthesis is indicated for a patient with PD who has pharmacologic therapy-refractory ED. Approximately 30% of patients presenting with PD have concomitant ED. In addition to placement of the prosthesis, further intervention may be needed to straighten the penis. One study noted that inflatable penile prostheses are associated with greater functional satisfaction and lower rates of persistent penile curvature deformity compared to malleable prostheses.[47] Guidelines for placement of penile prostheses for PD are available.[58] Intra-operative penile modelling is indicated if a penile curvature deformity >30° remains after prosthetic placement. Intra-operative 'modelling' of the penis over the inflated cylinders manually bent on the opposite side of the curvature for 90 seconds, often accompanied by an audible crack, has been introduced as an effective treatment.[59,60] If there is a residual curvature of <30°, no further treatment is recommended, as the prosthesis will act as a tissue expander and will result in complete correction of curvature in a few months.[59] If a residual curve >30° remains after modelling, then various techniques, including plaque releasing incision, are the next step. Grafting can be considered if tunical defects are >2.0 cm.[61,62]

Success rates with penile prosthesis implantation for PD range from 84–100%.[58,63,64] Partner satisfaction after placement of a penile prosthesis for PD was reported at 60.0–88.8%.[63] Complications associated with penile prosthesis include infection, loss of penile length, decreased penile sensitivity, malfunction of the prosthesis, erosion of the prosthesis, and persistent curvature.[48]

CONCLUSION

PD is estimated to affect 3–9% of men in the general population and is associated with significant negative effects on physical and psychosocial wellbeing, and quality of life. A variety of therapies have been used to treat PD with little available evidence to support their usage. Over the past 30 years, multiple modalities for the treatment of PD have come into view, and most have quietly disappeared. The early recognition of PD patients with their disease-specific issues will allow physicians to select the optimal treatment approach for their patients. More robust data from pooled analyses or databases at multiple high-volume centres would allow for a better retrospective review of various surgical and non-surgical outcomes.

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SIGNIFICANCE OF ASYMPTOMATIC BACTERIURIA

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ABSTRACT

Since the recognition of asymptomatic bacteriuria (ABU), several studies have questioned its significance. It is a very common condition, observed in many healthy individuals. Current guidelines mandate that ABU should not be treated in all cases, as it does not seem to improve the outcome. Conditional restrictions for treatment of ABU can be relaxed in certain situations, with minimal exceptions to the rule.

Keywords: Asymptomatic bacteriuria (ABU), culture, pyuria, significant bacterial count.

INTRODUCTION

The diagnosis of urinary tract infections (UTI) has a major role in its subsequent management. UTI are one of the most common infections reported in any hospital. Antibiotics are prescribed for most infections. Therefore, it is important to note that more often than not antibiotics are overprescribed, as is the case in the treatment of asymptomatic bacteriuria (ABU).[1] This short review will analyse the significance of ABU in adults with reference to the current guidelines and recommendations.

The role of the microbiology laboratory in the management of UTI is to support the clinical care through early, accurate diagnosis, with appropriate antimicrobial sensitivity testing. To date, the gold standard laboratory test for the diagnosis of UTI has been culture. The concept of ABU arose from the seminal work of Edward Kass,[2-4] who used criteria for assessing the significant bacterial count. A count of $\geq 10^5$ colony forming units (CFU) of bacteria per mL of urine was considered to be significant and to distinguish between the true pathogen and the indigenous flora. Later studies have shown that colony counts less than this can also be reflective of a true UTI.[5-7] Therefore, it is time we examined the reliability and applicability of this age-old concept in the laboratory diagnosis of UTI.

Current guidelines recommend us not to treat ABU, except in pregnancy and prior to most urologic surgeries where mucosal bleed is expected.[1,8] In a catheterised patient, it is difficult to determine whether bacteriuria signifies asymptomatic colonisation of the catheter or true infection. Most often the signs and symptoms of UTI are nonspecific or potentially attributable to another source of infection in these patients. It is noteworthy that in both non-catheterised and catheterised patients, the presence of pyuria is often considered an important finding.[1,9,10] Therefore, a combination of urinalysis and clinical presentation is needed to diagnose UTI.

PREVALENCE OF ASYMPTOMATIC BACTERIURIA

The term ABU refers to the isolation of bacteria in significant counts (>100,000 bacterial/mL) of a single bacterial species from a clean catch urine specimen of an individual who has no acute signs or symptoms. Though the detection of significant counts of bacteria in a clean catch midstream urine sample is practically acceptable, it has low credibility in women, unlike men, as the probability of a woman having true bacteriuria is only ˜80% with a single specimen. This probability increases to 95% if two or more consecutive cultures are positive for the same organism.[4,11,12] In the absence of other reliable modes of diagnosis, quantitative culture remains the gold standard for diagnosis of ABU in pregnancy.[13-15]

ABU is fairly common in the general population amongst adults, and as documented earlier,[10] is more common in women than in men, and increases with age.[8,16,17] ABU has been documented in 1–5% of premenopausal healthy women, which subsequently increases with age. Sexually active women tend to have a five-times higher prevalence than women who are not sexually active.[8,18,19] Compared to young women, ABU is uncommon in young men and, if present, signs of prostatitis should be looked for.[8] The reported prevalence rates of ABU in pregnancy range from 2–15%.[20] If left untreated, 20–30% of these may develop acute pyelonephritis by the second or third trimester.[18,21-24] ABU, which is otherwise a benign condition, is a cause for concern during pregnancy by most clinicians. Due to the growing fetus, the enlarged uterus impinges on the bladder, leading to urinary stasis and ureters, which cause hydronephrosis. This is compounded by the smooth muscle-relaxing effect of progesterone, which dilates the ureteric sphincter allowing the reflux of the urine into the renal pelvis, resulting in pyelonephritis which may have an ominous effect on the maternal, as well as the fetal, outcome. Among several other factors contributing to this, the most notable are glycosuria (due to gestational diabetes) and proteinuria in pregnancy-induced hypertension, both of which promote bacterial growth. Bacterial products, namely endotoxins, can result in secretion of pro-inflammatory cytokines by either the maternal or fetal macrophages, leading to precipitation of labour.[20-24] ABU in postmenopausal women varies from ˜5–19%, and is much more common in women with a prior history of UTI.[8,18,25] There are many contributing factors, which include a diminishing oestrogenic effect on the genitourinary mucosa, urologic abnormalities such as cystoceles, gynaecological abnormalities like genitourinary prolapse, any surgical manipulations on the genitourinary tract, and genetic predisposition.[26] In elderly men, the prevalence of ABU ranges from 4–7%; the major predisposing factor is prostatic enlargement leading to bladder outlet obstruction.[27] With increasing age, dementia, and decreased mentation, impaired bladder voiding, and incontinence of the bladder and bowel, compound the existing structural and functional impairments, resulting in a higher frequency of bacteriuria.[27] Contrary to some earlier studies,[28,29] ABU in the elderly is not associated with increased mortality.[10] The prevalence of ABU is also found to increase with associated co-morbidities, like diabetes mellitus.[17,30-32] In individuals with spinal cord injury, there is incomplete bladder emptying, which promotes bacterial growth, thereby resulting in bacteriuria.[31,33] ABU has been found to be higher in the early post-transplant period in cases of renal transplant recipients.[31] Moreover, immunosuppressants can mask the signs and symptoms of infection, making distinguishing between ABU and a true infection difficult.[8,31]

PATHOGENESIS OF ASYMPTOMATIC BACTERIURIA AND THE ROLE OF MICROBIAL FLORA

There are many factors which can predispose an adult to having ABU, including the genetic composition of the host, the presence of a foreign body, incomplete bladder emptying, any prior instrumentation, etc. In the elderly, the reasons for a higher prevalence of ABU are multifactorial. Due to the reduced immune response in the elderly, this colonisation tends to be persistent and more so in diabetics, as documented earlier.[34-38] Neurogenic bladder and resulting incontinence, obstructive uropathy, altered bladder mucosal defences and reduced effective cell-mediated immunity, alterations of urinary and vaginal pH as a result of declining hormonal secretions, and glycosuria, can result in increased colonisation, due to bacteria in the urogenital tract.[17,34-38] Glycosuria encourages the growth of bacteria *in vitro*, but it is not well ascertained whether ABU is a precursor of symptomatic bacteriuria in patients with diabetes.[39-41] ABU does not lead to severe complications in diabetes; hence, routine screening is not warranted in these individuals.[42] Certain genetic polymorphisms in the innate immune receptors that downregulate the immune response, for example the TLR4 promoter region, have been linked to ABU state. Conversely, increased levels of interleukin (IL)-8 have been observed in individuals with ABU. This increase is, in turn, triggered by the binding of the fimbriae and the lipopolysaccharides to the specific TLR4.[43,44] This activation causes recruitment of the neutrophils, which aids in localising the organism within the bladder and prevents it from ascending.

The commensal flora of the periurethral area, vagina, or the gut are usually the source of the bacteria isolated from the urine of patients with ABU. Most often (80–90%), the organism is *Escherichia coli*, followed by *Enterococcus*, *Klebsiella*, and *Proteus*. A variation in the culture isolation of the organisms depends on the patient

characteristics; namely, diabetes, female sex, pregnancy, whether they are catheterised, old age, etc.[45] The organism can exhibit many relationships with the host in various forms, either as a commensal, coloniser, or a pathogen. It is interesting to note that there are strains of *E. coli* which have been isolated from cases of ABU. Studies have documented that strains of *E. coli* colonising the urothelial cells in these patients are less virulent,[46-49] the best studied being *E. coli* 83972, and those that have been isolated from symptomatic UTI cases are identified as uropathogenic *E. coli* (UPEC), the best characterised being UPEC CFT073. Conversely, UPEC strains have also been isolated from cases of ABU, as otherwise ABU strains could not be differentiated from those causing cystitis or pyelonephritis.[50-52]

Using comparative genomics, it was noted that *E. coli* 83972 and UPEC CFT073 were phylogenetically related and had similar origins. The former evolved from a pathogen to become a commensal, shedding its virulent characteristics. Certain point mutations have been detected in the gene expressing the papG fimbria (because of which the P fimbriae can no longer adhere to its receptor) and in foc D, which is located in the outer membrane, behaving like a usher protein for F1C fimbriae, thus rendering it incapable of reaching the cell surface.[47,53] Moreover, this evolution is driven by host factors, which have been observed in many studies but are not well understood.[54] Notably, there are many adhesins that aid in the colonisation of both UPEC and ABU strains of *E. coli,* but none act in isolation; it is always a combination of these adhesive factors that culminate in symptomatic infection or an ABU. Hence, there are many more factors that may be responsible in the variation of gene expression of this organism, and which are responsible for the outcome of the disease state of the host; either an ABU or a symptomatic infection.

SAMPLING OF URINE

The most practically feasible and routinely submitted sample for a culture and urinalysis for diagnosis of UTI is the midstream clean catch urine, which is collected early in the morning. The sample, if collected in this manner, is likely to give a clear picture of the contents of the urine, which in turn forms an approximate reflection of the ongoing processes in the urinary tract.[55] Errors arise due to the improper cleaning of the skin and surrounding areas while collecting urine. The use of catheterisation to collect samples is not advisable either, as the skin contaminants may gain access to the urinary tract, which can be harmful, and the distal urethral microbial flora can be allowed to ascend, which can result in a UTI.[56]

In cases where the patient is catheterised, the sample can be easily obtained from the sampling port of the catheter after cleaning it using appropriate, routinely used, antiseptics. A suprapubic puncture is by far the ideal sample, as it is collected directly from the bladder under strict aseptic precautions,[56,57] but, being an invasive procedure, is rarely opted for unless indicated.

The sample collected by any of the above means should be transported to the laboratory within 2 hours of collection or should be stored under refrigerated conditions (2–8°C) for a maximum of 24 hours. In cases of delay, some preservatives, such as boric acid (1.8%), sodium chloride-polyvinyl pyrrolidone, and boric acid-glycerol-sodium formate, can be used, but there are problems inherent to the use of such agents, as described previously.[6,55-57]

Considering the prevalence estimates of ABU in different groups, the recommendations for screening of these same groups applies with conditions. The screening may not be beneficial in certain groups where the prevalence is high and treatment does not have any effect on the outcome of ABU (Table 1).[8,35,50,58]

INTERPRETING URINALYSIS AND INTERPRETING URINE CULTURES

The significance of colony count varies depending on a number of factors, including the patient, effect of antimicrobial therapy, stage of infection, fluid intake/frequency of micturition, underlying illness, presence of a urinary catheter, age and sex, pH of urine, growth rates of the organisms, site of infection, and residual urine, etc.[10,17] Growth of a single organism in significant counts is to be considered in cases of ABU in pregnancy and prior to urological surgeries, for the reasons stated above.[10,17] Isolation of more than one organism on culture reflects contamination in a midstream clean catch urine to more accurately reflect the fact that co-infection or multi-species infections are uncommon but do occur given the overall burden of ABU, and therefore avoids misinterpretation. In general, two consecutive urine samples in women need to be analysed for the diagnosis of ABU.[10]

Table 1: Recommendations for screening of asymptomatic bacteriuria.

Who should be screened	Who should not be screened	No specific guidelines available
Pregnant women	Diabetic women	Post-renal transplant
People prior to any urological surgery	Elderly males or females	Neutropenia
	Those with a spinal cord injury	
	Those with a neobladder	
	Those with an ileal conduit or who have undergone ilealcystoplasty	
	Catheterised individuals	

More often than not, in most of these cases, the follow-up sample may not match the initial positive culture result, which is indicative of a contaminated specimen. In the case of catheterised urine, a threshold of ≥1,000 CFU/mL is considered significant, though in many centres even lower CFU is considered significant.[10] In such cases, the sampled organisms may be from biofilms on the inner surface of the catheter; hence, culture reports of catheterised urine may not accurately reflect bladder bacteriuria.

Pyuria is often reflective of an ongoing UTI, especially upper UTI, but it is not specific to UTI.[9,59,60] The presence of pyuria is not necessarily linked to inflammation due to infection in the urinary tract. It has also been noted in healthy individuals, including schoolgirls, women with ABU, and in those with chronic indwelling catheters. Very often, individuals with ABU do not have pyuria, in which case the rapid tests to detect leucocyte esterase will be negative. Thus, pyuria has a limited role to play in the context of ABU; its presence or absence should not influence the use of antibiotics. Also, one needs to note that rapid dipstick tests to detect nitrite, as well as the leucocyte esterase, have several limitations of their own. Thus, they are not used for diagnosing ABU.[50,61]

ROLE OF TREATMENT

ABU is a common clinical finding. It may be observed in otherwise healthy individuals, as well as in those with functional or morphological defects of the genitourinary tract, the burden of ABU being higher in the latter. This creates a dilemma among clinicians and health professionals as to whether or not to treat this condition.

Guidelines strictly recommend screening and treatment of ABU in cases of pregnant women, to reduce chances of pyelonephritis and reduce associated fetal damage.[10,17] The isolation of *Streptococcus agalactiae* from the urine of a pregnant woman also needs to be treated with antibiotics, because of the potential risk to the baby during vaginal delivery.[45] Surprisingly, despite the large amount of literature available, there is little evidence to support the fact that treatment of ABU in pregnancy actually reduces the risk of preterm labour.[8,50,60] Also, most of the existing guidelines do not mention the duration of therapy of ABU during pregnancy. Guidelines also recommend screening and treatment of ABU prior to urological interventions, as surgery causes mucosal injuries which allow these bacteria to penetrate the tissue, causing local infection, and can even gain access to the blood circulation resulting in bacteraemia.[8] Studies have shown no benefit in treatment of ABU in patients with an ileal conduit, ileocystoplasty, or orthotopic neobladder, and patients using clean intermittent catheterisation, as they frequently become colonised and it is difficult to eradicate this colonised flora using antibiotics.[8,50] Antibacterial treatment of ABU in diabetic patients failed to reduce the risk of symptomatic UTI and infectious complications, while untreated ABU did not correlate with any increase in complications. In such cases, it is important to understand that with good control of diabetes itself the risk for symptomatic UTI and infectious complications can be reduced.[8,50]

Cohort studies, as well as placebo-controlled trials, involving spinal cord injury patients have not shown any decrease in symptomatic infection when antibiotic therapy for ABU was used. Reports show that low urinary bladder pressure aids in preventing renal failure in the presence of ABU in such cases.[61] Fiorante et al.[62] reported that there were no differences in the prognosis of the

renal allograft among those who developed ABU after transplant and those who did not, though treatment of ABU may have had an impact on reducing the incidence of pyelonephritis in post-renal transplant recepients.[63]

The challenge lies in dealing with patients admitted to intensive care units who tend to have ABU due to prolonged catheterisation. In such patients, it is difficult to differentiate between colonisation or true infection. Also, in neutropenics and transplant individuals, the relevance of ABU is still unclear, as this could be either a colonisation or a focus of invasive infection, though this could possibly be a risk factor for patients with ABU to develop symptomatic UTI.[50] In the absence of considerable evidence that antibacterial therapy of ABU can reduce infectious complications in such cases, treating with antibiotics is still considered a viable option for many clinicians. More prospective studies are needed to shed light on these aspects.

Several studies to date have clearly shown that antimicrobial treatment of ABU does not reduce the frequency of ABU. Prospective cohort studies, as well as randomised controlled studies, including patients with spinal cord injury, patients with diabetes, non-pregnant women, elderly people, and individuals with chronic catheters, have failed to show any benefits of treatment of ABU. In fact, such unwarranted treatment has resulted in subsequent isolation of resistant bacteria,[10,17,64] collateral damage in terms of alteration of the normal gut flora resulting in increased risk of *Clostridium difficile* infection, and even increasing the risk of UTI by destroying the harmless colonised flora in the genitourinary tract.[10,50,65] Notable observations made from many prospective randomised controlled trials indicate that eradication of these less virulent strains have led to pyelonephritis and in some cases recurrence of UTI.[49,65-67] The hypothesis that such colonised, less virulent, strains offer some kind of protection by preventing virulent strains from causing infection has paved the way for the concept of 'bacterial interference'.[49,68] It needs to be emphasised here that untreated ABU is not harmful and rarely causes renal failure. Any episode of bacteriuria does not necessarily confirm a true UTI and, hence, should not be treated unless warranted. Any antibiotic exposure will eventually contribute to the phenomenon of antibiotic resistance. Hence, the use of antibiotics should be minimised through regular dialogue between the laboratory and the clinicians concerned, with emphasis on the idea of choosing wisely.[69]

In conclusion, for any bacteriuria to remain as ABU or a symptomatic UTI is determined by a complex interplay of organism, host, and environmental factors. Certain conditions with ABU continue to be a dilemma for most healthcare professionals. Nevertheless, caution needs to be exerted while treating most of them wherever clear-cut guidelines are not available. Unwarranted treatment of ABU with antibacterials contributes to antibiotic resistance.[18] Therefore, it is important to raise the levels of awareness regarding ABU among healthcare professionals and, even more, to implement restrictions over the use of antibiotics for treatment of ABU.

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THE ROLE OF SELECTIVE SEROTONIN REUPTAKE INHIBITORS IN PREMATURE EJACULATION

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ABSTRACT

Premature ejaculation is a very common male sexual medical illness worldwide. Amongst several biological risk factors, the disturbance of central serotonin neurotransmission is one important cause. Various research has allowed a better understanding of the development of this disorder, as well as allowing for improved treatment options in order to decrease the significant morbidity associated with this condition; among the more recent treatments, selective serotonin reuptake inhibitors are believed to be one of the best. During this review, the action and role of serotonin reuptake inhibitors at different levels will be discussed, along with their relevance and effectiveness in the treatment of premature ejaculation.

Keywords: Dapoxetine, fluoxetine, premature ejaculation (PE), selective serotonin reuptake inhibitors (SSRI), serotonin, 5-HT receptors.

INTRODUCTION

Premature ejaculation (PE) was once considered to be a psychosomatic disturbance.[1] This sexual disorder is more common than some estimate and is responsible for significant emotional and psychological issues.[2] Researchers have faced difficulties studying PE and physicians still do not know the most effective treatments, since patients are often unwilling to divulge personal information.[3,4] According to Guiliano and Hellstrom,[5] lack of knowledge of aetiology and lack of approved treatments may be responsible for under-diagnosis and under-treatment of PE. PE can be a lifelong condition (PE present since the onset of sexual maturity) or acquired (PE is secondary to other conditions such as chronic prostatitis, diabetes mellitus, and hyperthyroidism). On treating the underlying pathology, it is possible that acquired PE can be reversed.[6]

Although many definitions of PE have previously been described, and the definitions given in the Diagnostic and Statistical Manual of Mental Disorders (DSM) by the American Psychiatric Association (APA) were largely accepted by the medical community with little discussion, these definitions have no evidence-based medical support.[7] The first evidence-based unified definition for both acquired and lifelong PE was given by the first Ad Hoc Committee of the International Society for Sexual Medicine (ISSM) for the Definition of Premature Ejaculation. The committee stated that lifelong PE was a male sexual dysfunction that was determined by the presence of three criteria: i) ejaculation always (or nearly always) occurring either prior to vaginal penetration or within 1 minute; ii) inability to delay ejaculation in all (or nearly all) vaginal penetrations; and iii) negative personal consequences for the affected individual, including distress, frustration, bother, and/or avoidance of sexual intimacy. This definition was then expanded upon in 2013 by the second Ad Hoc ISSM Committee for the Definition of Premature Ejaculation. The committee noted that an additional characteristic by which acquired PE could be defined was the presence of a clinically significant and bothersome reduction in latency time (to about 3 minutes or less).[7]

EJACULATION AND THE EJACULATORY NEURAXIS

Ejaculation is a complex sexual response consisting of two sequential phases: emission (deposition of seminal fluid and sperm in the posterior urethra) and expulsion (propulsion of the ejaculate out of the urethra). The whole process occurs through the interaction of supra-spinal, spinal, and peripheral neural pathways. The spinal part is a result of a reflex, requiring sympathetic, parasympathetic, and somatic efferent pathways.[8] The cell bodies of primary sensory neurons innervating peripheral anatomical structures participating in ejaculation are located in lumbar dorsal root ganglia, and their central projections terminate in the medial dorsal horn and the dorsal grey commissure (Lamina VII and X) of the T10–L2 spinal cord segments.[9] In humans, functional magnetic resonance imaging studies revealed that in cases of PE, the brain responses and functional integration in certain brain areas were impaired.[10] The hindbrain nucleus paragigantocellularis (nPGi) projects bilaterally to the lumbosacral motor neuron pools that innervate the genital musculature. nPGi lesions facilitate ejaculation, proving that nPGi is the source of descending inhibition to genital reflexes.[11,12] When men are sexually stimulated, neurological signals are sent to the spinal cord and brain. At a certain level of excitement, signals are then sent from the brain to the reproductive organs.[13] This causes semen to be released through the penis. Pharmacological delay of ejaculation can be achieved either by inhibiting excitatory or reinforcing inhibitory pathways from the brain or from the periphery to the spinal cord.[8]

Many neurotransmitters and receptors are found at the ejaculatory neuroaxis including dopamine, nitric oxide, and 5-hydroxytryptamine (5-HT), also referred to as serotonin.[8,9] Dopamine causes excitation of the ejaculatory neuroaxis, and inhibition occurs by serotonin and nitric oxide.[9] All serotonin receptors are located post-synaptically, except for 5-HT1A, 5-HT1B, and 5-HT1D which are pre-synaptic and involved in negative feedback.[9] These 5-HT receptors are found throughout the central and peripheral nervous system, from the brainstem, hypothalamus, nPGi, and the dorsal horns of the spinal cord, as well as in structures involved in ejaculation, including the seminal vesicles, vas deferens, urethra, and prostate. Serotonin induced contraction of urogenital organ smooth muscle could regulate the emission of urine and/or semen.[9,14-16] Disturbance of the central (spinal and supra-spinal) serotonergic neurotransmission is principally responsible for ejaculation. Presynaptic 5-HT1B and postsynaptic 5-HT2C receptor stimulation is thought to increase ejaculation latency time and 5-HT1A receptor stimulation is also known to play a role in ejaculation.[17]

DISCUSSION

Action of Selective Serotonin Reuptake Inhibitors in Premature Ejaculation

Serum 5-HT levels could be used as a diagnostic tool for PE and as an indicator in PE treatment.[18] Selective serotonin reuptake inhibitors (SSRI), such as paroxetine, fluoxetine, and sertraline, are used to treat depression and mental health disorders and can cause delayed or blocked ejaculation as a side effect in men. Clinical studies have shown that after treatment with SSRI, intravaginal ejaculation latency time gradually increased. Therefore, SSRI have opened a new potential avenue for PE treatment.[19]

Due to their pharmacokinetic profile and pharmacodynamic activity, these agents are intended for chronic use. SSRI actively block presynaptic membrane 5-HT transporters and thus inhibit the 5-HT (serotonin) reuptake and breakdown; this results in availability of higher levels/activity of serotonin in the synaptic cleft. Increased synaptic availability of serotonin facilitates its binding to 5-HT receptors, leading to a delayed ejaculation.[17] SSRI induced inhibition of ejaculation may be mediated by the serotonergic ventrolateral periaqueductal gray nPGi pathway.[20]

Dapoxetine Treatment

Dapoxetine is a short acting oral SSRI, purely created for the on-demand treatment of PE.[8] Dapoxetine cannot permanently cure PE but has increased in importance due to fewer side effects, as a result of its rapid absorption, fast action, and fast excretion. Dapoxetine inhibits the reuptake of serotonin, dopamine, and noradrenaline transporters. Nausea, dizziness, and headache were the most commonly reported side effects.[21-23] However, the US Food and Drug Administration (FDA) issued a non-approval letter for dapoxetine in 2005, and approval is still anticipated.[24]

Dapoxetine has a much lower ejaculation-delaying effect compared with traditional SSRI. Fold increase in mean geometric intravaginal ejaculation latency time with dapoxetine on-demand treatment was

approximately three-fold, versus an estimated nine-fold increase with daily paroxetine, five-fold increase with daily clomipramine, and four-fold increase with daily sertraline and fluoxetine. Chronic treatment with SSRI does, however, produce unwanted, adverse sexual effects as well as withdrawal symptoms upon abrupt discontinuation. The activation of presynaptic 5-HT1A auto-receptors and chronic 5-HT1A auto-receptor desensitisation may be a contributing factor to an increase in the number of side effects and withdrawal symptoms.[5] In young adults, suicidal thoughts and behaviour were also seen, which limits their use. There are also a few case reports indicating that these sexual side effects may continue beyond cessation of SSRI treatment; these would, theoretically, be reduced by on-demand use of SSRI (such as dapoxetine). While discontinuation syndrome was more common with short half-life SSRI, dapoxetine treatment was associated with a low incidence of discontinuation syndrome. The safety profile of dapoxetine is good but the safety of its long-term use is currently unknown.[17,25-27] The American Urological Association (AUA) guidelines state that the relative effectiveness of daily dosing SSRI and on-demand SSRI in the treatment of PE is inconclusive. The lowest possible therapeutic doses of oral antidepressants are compatible and successful at helping to treat PE. According to the European Association of Urology (EAU) guidelines, daily treatment with SSRI has become the first-choice treatment in PE.[17]

It is postulated that on-demand (acute) treatment with SSRI, such as dapoxetine, will not produce an ejaculation delay equivalent to daily long-term (chronic) treatment of SSRI, such as fluoxetine and paroxetine. This may be due to the discontinuous use of on-demand SSRI, which does not produce a continuous elevated level of serotonin in testis and accessory reproductive organs[28-30] and can produce histological changes in such reproductive organs in the form of proliferation of seminal vesicle mucosal crypts, narrowing of lumen, and change in mucosal lining from secretory mucosa to non-secretory low cuboidal and to flat cells.[28] Due to there not being any physiological impairment as a result of PE, any pharmacological agent with either a central or peripheral mechanism of action that delays ejaculation could therefore be a potential therapeutic drug candidate for the treatment of PE.[8]

CONCLUSION

The benefits of any pharmacotherapy should, as always, be weighed-up against their safety and efficacy. SSRI appear to be the most important therapeutic drug for PE with regard to their multiple sites of action in the regulation of the complex mechanisms involved in ejaculation. There is a need for further experimental and clinical studies to fully understand whether the histological changes produced by low doses of chronic SSRI in seminal vesicles are permanent and responsible for relieving or curing the symptoms of PE; it may be that these doses of SSRI could be curative for PE sufferers. Use of low to moderate therapeutic daily doses of SSRI for chronic use/long duration for the treatment of PE could reduce the frequency and severity of adverse PE events.

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DO PREOPERATIVE ALPHA BLOCKERS FACILITATE URETEROSCOPE INSERTION AT THE VESICO-URETERIC JUNCTION? AN ANSWER FROM A PROSPECTIVE CASE-CONTROLLED STUDY

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Ethical approval: Ethical approval has been taken from The Institutional Ethical Committee. Informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.
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ABSTRACT

Objective: To evaluate whether alpha blockers facilitate the navigation of the ureteroscope through the ureteric orifice, if administered preoperatively, based on the role of alpha blockers, mainly tamsulosin, in medical expulsive therapy of ureteric calculus.

Methods: A prospective, case-control study of 174 patients who underwent ureteroscopic stone removal for lower or mid ureteral calculi between November 2014 and March 2016 was carried out. We included patients >14 years of age who were planned for ureteroscopic stone removal. We divided the patients into two groups, including those who were not prescribed alpha blockers prior to surgery (Group A: no alpha blocker) and those patients who were started on alpha blockers, tamsulosin 0.4 mg, 3 days prior to surgery (Group B: alpha blocker). We excluded patients with stone size >1 cm, spontaneous stone passage prior to surgery, unable to perform ureteroscopy (URS), and previous history of ureteroscopic intervention.

Results: Our study included 124 patients, among whom 60 patients were prescribed alpha blockers preoperatively (Group B). The mean age of the study population was 37.62+9.74 (15–64 years) and the mean stone burden was 38.92+8.21 (15.94–58.12 mm^2). The difference in rate of ureteroscope negotiation through the ureteric orifice between Groups A and B was not statistically significant (p=0.57).

Conclusions: In URS, preoperative administration of alpha blockers failed to improve technical ease and lower complication rate. Further large group, multi-centre studies are required todiscover a definitive role of alpha blockers prior to URS.

Keywords: Urolithiasis, ureteral stones, medical expulsive therapy (MET), alpha blockers, tamsulosin, ureteroscope.

INTRODUCTION

Urolithiasis is a worldwide public health problem. The estimated prevalence is 8–13% in the USA, 5–8% in Europe, and 1–5% in Asia.[1] Urolithiasis is one of the most common reasons for frequent visits to hospital emergency departments. Ureteral stones are the leading cause of colicky pain and

the majority of these pass spontaneously. In the literature, it has been reported that ~70% of ureteral calculi were located in the lower third part of the urinary tract.[2] Medical expulsive therapy (MET) is recommended by the European Association of Urology (EAU) (2013) for 5–10 mm ureteric stones to facilitate stone passage. For MET, alpha blockers, mainly tamsulosin, have shown efficacy in several randomised controlled trials.[3-5] The underlying pathophysiology of this therapy is supported by the presence and distribution of adrenoreceptors in the ureter; alpha-1D and alpha-1A are the most common adrenoreceptors, and are more numerous in the distal ureter.[6] Blocking the action of alpha-1 receptors by pharmacological agents (alpha blockers), such as alfuzosin, terazosin, doxazosin, and, most typically, tamsulosin, results in the relaxation of the ureteric smooth muscle.[7-9]

Ureteroscopy (URS) is the most commonly performed procedure for the treatment of ureteral calculi, with a high (>90%) stone-free rate after a single treatment. Technical advancements in the armamentarium of semi-rigid URS, including the design and miniaturisation of scopes and availability of the Holmium:YAG laser, ensures a precise and excellent safety profile with a powerful stone fragmentation mechanism.[10] Difficulty in negotiating a ureteroscope or ureteral access sheath through the vesico-ureteral junction is a common occurrence. Surgeons advocate various strategies to facilitate the passage of ureteroscope or ureteral access sheath for performing URS or retrograde intrarenal surgery. Routine preoperative stenting has been recommended by some authors to facilitate passive ureteral dilatation.[11]

Based on the role of alpha blockers, mainly tamsulosin, in MET of ureteric calculus, we attempted to extend the use of alpha blockers prior to URS for procedural ease. We conducted a prospective, case-controlled study to evaluate whether alpha blockers facilitate the negotiation of the ureteroscope if administered preoperatively.

MATERIALS AND METHODS

Study Design

We conducted a prospective, case-controlled study of 174 patients at the Urology Department, King George's Medical University, Lucknow, Uttar Pradesh, India, who underwent ureteroscopic stone removal for lower or mid-ureteric calculi between November 2014 and March 2016. We included patients >14 years of age who were planned for ureteroscopic stone removal. We divided the patients into two groups: those who were not prescribed alpha blockers prior to surgery (Group A: no alpha blocker [NAB]) and those patients who were started on alpha blocker tamsulosin 0.4 mg, 3 days prior to surgery (to decrease the chances of under dosing and to achieve optimum serum level) (Group B: alpha blocker [AB]). We excluded patients with stone size >1 cm, spontaneous stone passage prior to surgery, those who could not have URS performed, patients with ureteral strictures and ureteral anomalies, and previous history of ureteroscopic intervention. Eighteen patients were excluded from the study due to consent withdrawal (5 patients), spontaneous stone passage (2 patients), and failure to perform the procedure (procedure was abandoned due to inaccessible ureteric orifice) (11 patients). Patients were divided into either the NAB group or AB group (Figure 1).

Enrolled patients were interviewed about their age, sex, occupation, medical history about stone-related symptoms, previous treatment, urinalysis, urine culture, x-ray KUB (kidney, ureter, and bladder), ultrasound KUB, excretory urogram, or non-contrast computed tomography KUB (NCCT-KUB). All patients were treated preoperatively as per a urine culture report. We calculated stone size by multiplying the two largest dimensions on available radiological study (x-ray KUB, ultrasound KUB, excretory urogram, or NCCT-KUB). Intraoperative and postoperative data, including stone clearance and complications, were recorded (Table 1).

Operative Technique

At our centre, semi-rigid URS (6.5/8.5 French scale [Fr], Wolf, Richard Wolf Medical Instruments Corporation, Illinois, USA) is performed by standard technique by experienced urologists, all senior residents and faculty of the department, and under regional or general anaesthesia. No significant difference in anaesthesia technique was made between the two groups. Operative time was calculated from insertion of the ureteroscope per urethra to ureteroscope removal after stone removal. The ureteric orifice was dilated with the help of a balloon dilator (8 Fr), ≤14 atmospheric pressure for 2 minutes under direct vision with a 22 Fr cystoscope, if required. Stone fragmentation was performed either using a holmium laser (365 nm fibre, Auriga holmium laser system, StarMedTec GmbH, Starnberg, Germany) or pneumatic lithotripter, based on the surgeon's preference or availability of equipment.

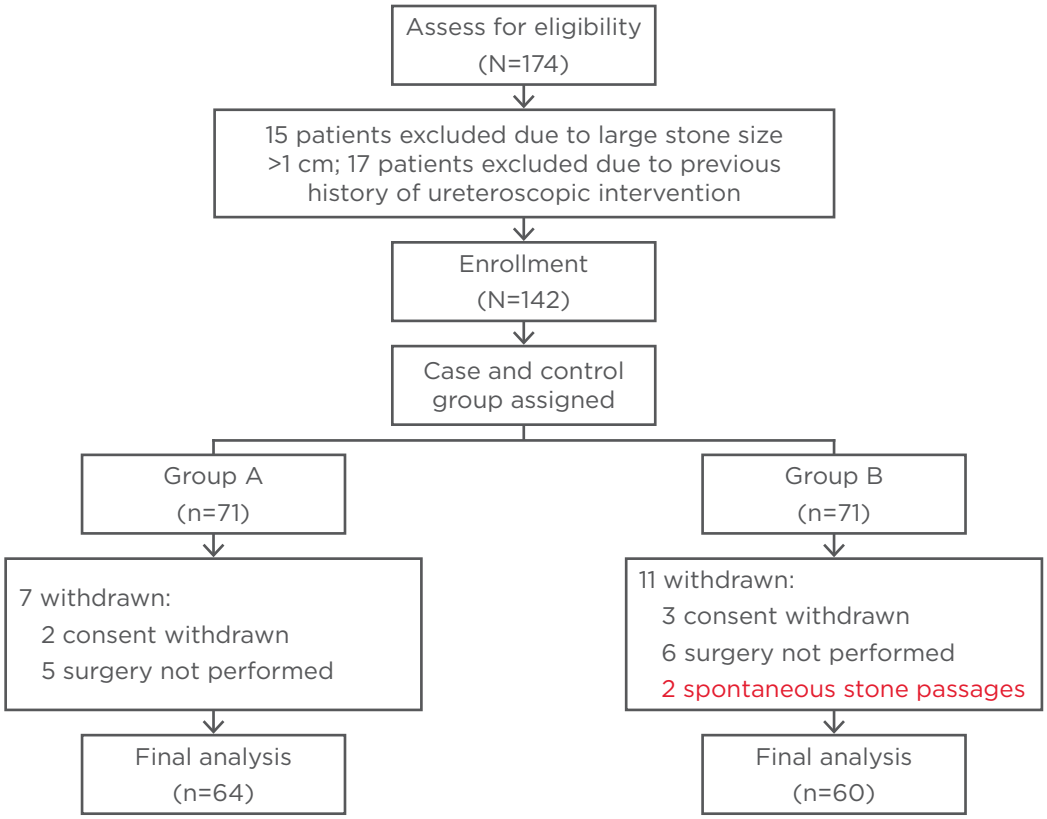

Figure 1: Schematic diagram showing study design.

Fragments of stone were removed with the help of removal forceps. On completion of the procedure, we placed the 5/6 Fr double J stent under fluoroscopy (Siemens Multimobil 5E system, Siemens Healthcare GmbH, Erlangen, Germany) in selected cases. Foley catheter was removed on postoperative Day 1. Postoperative clearance was checked by imaging, including x-ray KUB or USG KUB on postoperative Day 1. The double J stent was removed after 1–2 weeks under sedation and antibiotic coverage. All complications were reported by the operating urologist using the Modified Clavien Classification System. A separate grading protocol was used for the patients who had >1 complication, with each one reported separately.

Statistical Analysis

Data analysis was conducted using SPSS Statistics version 16.0 (Chicago, Illinois, USA). The unpaired t-test was used for analysing continuous variables, and categorical or dichotomous variables were evaluated by using the chi-square test. The results were presented as a proportion (%) and mean (+ standard deviation). All p values were two-pronged, with $p<0.05$ considered statistically significant.

RESULTS

Our final analysis included 124 patients, among whom 60 patients were prescribed alpha blockers preoperatively (Group B). The mean age of the study population was 37.62+9.74 (15–64 years). The mean stone burden was 38.92+8.21 (15.94–58.12 mm^2) including 77.42% population with lower ureteric stones. Out of 124 patients, 41 (33.06%) patients did not require vesico-ureteric junction dilatation for ureteroscope insertion and 60.98% were prescribed alpha blockers preoperatively. The difference in the rate of ureteroscope negotiation through the vesico-ureteric junction between the NAB and AB groups was not statistically significant (p=0.57) (Table 1).

DISCUSSION

In the human ureter, three types of alpha-1 adrenergic receptors are expressed, among which subtype alpha-1D predominates.[11] Experimental studies showed that the alpha-1A adrenergic receptor has a key role in ureteral contraction.[12,13] Tamsulosin effectively blocked all subtypes of alpha-1 adrenergic receptors and inhibited spontaneous ureteral contractions. In the literature,

Level I evidence exists, supporting the role of alpha-1 adrenergic receptor antagonists in conservative management of ureteral stones.[14,15] The underlying mechanism of stone expulsion is most likely related to direct inhibition of ureteral contractions and subsequent ureteral relaxation.

Patients with ureteric calculus perceive anxiety about the sudden onset of colicky pain and remain uncertain about spontaneous passage of stones. Several meta-analyses and randomised controlled trials encouraged clinicians to prescribe MET to decrease stone-related symptoms, facilitate and hasten stone passage, and reduce the need for active intervention. In the current scenario, an alpha-blocker (tamsulosin 0.4 mg once daily) and a calcium channel blocker (nifedipine 30 mg once daily) have shown efficacy in the production of desired clinical outcomes in several studies.[16,17]

Ureteroscopic lithotripsy has emerged as a favoured procedure for ureteric calculus by a urologist, because of the high stone-free rate and immediate symptom alleviation. In cases of difficult ureteral access, the ureteric orifice has to be dilated, either by balloon dilator or similar means. The use of balloon dilators may be associated with significant ureteral injury.[18]

In our study, we divided and assigned patients based on administration of daily doses of tamsulosin 0.4 mg prior to URS. Out of 124 patients, 33% of patients did not require ureteric orifice dilatation for ureteroscope insertion.

Table 1: Pre and postoperative data of ureteroscopy procedure.

Variable	Total	NAB group (A)	AB group (B)	p value
Number of patients	124	64	60	
Male/female	71/53	38/26	33/27	1.00
Right/left	59/65	30/34	29/31	1.00
Patient age (years) Mean±SD (Range)	 37.62±9.74 (15–64)	 38.12±7.31 (15–63)	 37.26±8.75 (16–64)	0.55
Preoperative serum creatinine Mean±SD (Range)	 1.04±0.2 (0.64–2.10)	 0.96±0.31	 1.00±0.07	0.33
Stone burden (mm^2) Mean±SD (Range)	 38.92±8.21 (15.94–58.12)	 36.86±9.32 (16.97–58.12)	 39.74±8.93 (15.94–57.64)	0.08
Stone location Middle (%) Lower (%)	 28 (22.58) 96 (77.42)	 13 (20.31) 51 (79.68)	 15 (25.00) 45 (75.00)	0.52
Comorbidities Diabetes mellitus Hypertension Systemic steroid use	 7 6 1	 5 3 0	 2 3 1	
Ureteroscope negotiation Without VUJ dilatation With VUJ dilatation	 41 (33.06) 83 (66.94)	 16 (39.02) 48 (57.83)	 25 (60.98) 35 (42.17)	0.57
Complications (%)	26 (20.97)	17 (26.56)	9 (15)	0.13
Grade I Mucosal injury Fever Haematuria Grade II Urinary tract infection Grade III Perforation Grade IV Grade V	 7 6 7 5 1 - -	 4 4 5 3 1 - -	 3 2 2 2 0 - -	

NAB: no alpha blocker; AB: alpha blocker; SD: standard deviation; VUJ: vesico-ureteric junction.

Among these 41 (33%) patients, 25 (61%) belonged to Group B in which tamsulosin was started as compared to 16 (39%) from Group A. This difference was statistically insignificant, but clinical application in reducing the need of ureteral dilatation during URS can be appreciated by the operating urologist in reducing operative time and complications. The pathophysiological basis of using alpha blockers in MET for ureteric calculus may have hidden potential which can be applied in other ureteral interventions. Our study is an effort to reveal the effect of alpha blockers on ureteral orifices and the lower ureter, which may facilitate the URS and help to reduce procedural complexities.

Our study is strengthened by being one of the first studies in which pre-URS alpha blockers were used. The limitations of this study included the heterogeneity of surgeon's experience, lithotripsy (holmium and pneumatic), and anaesthesia (general versus spinal). Moreover, our study is limited by the small number of subjects. To the authors' best knowledge, no such study is found in the literature. We conducted a prospective, case-controlled study to reduce selection bias.

URS is a safe and effective procedure. In the literature, a complication rate ranging between 3%[19] and 30% has been described. Mandal et al.[20] studied 120 patients who underwent unstented URS and reported a 30% complication rate. The Modified Clavien Classification System has classified complications in five grades. Grades 1 and 2 are considered as minor and Grades 3–5 as major complications. In our study, we encountered an overall complication rate of 21% (77% minor). No statistically significant difference in complication rate was found between the two groups.

CONCLUSION

Ureteroscopic lithotripsy is a safe and effective procedure for ureteric calculus with the highest stone-free rate and immediate symptom relief. The use of alpha blockers, mainly tamsulosin, is well-proven in MET. In URS, preoperative administration of alpha blockers failed to demonstrate the benefits of technical ease and lower complication rate. Further large group, multi-centric studies are required to find a definitive role for alpha blockers prior to URS.

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CONTEMPORARY EVIDENCE, TREATMENT STRATEGIES, AND INDICATIONS FOR CHRONIC TOTAL OCCLUSION-PERCUTANEOUS CORONARY INTERVENTION

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ABSTRACT

Chronic total occlusions (CTOs) are detected incidentally in ~20% of patients undergoing coronary angiography and are often associated with significant morbidity and mortality. CTOs can manifest with worsening symptoms, reduced left ventricular function, and increased incidence of ventricular arrhythmias. Despite this, according to USA, Italian, and Japanese national registry data, only ~5–22% of CTO lesions are treated by percutaneous coronary intervention (PCI). CTO-PCI is a particularly challenging technique for this subset of lesions and has traditionally been associated with increased risks and complications compared to conventional PCI. However, increased experience, the development of novel techniques, and dedicated equipment have revolutionised CTO-PCI. USA, Italian, and Japanese registry data have shown success rates of between 85% and 90%, with diminishing complication rates when performed by experienced operators. Moreover, observational studies have suggested that there are significant benefits of using CTO-PCI, including fewer symptoms, improved quality of life, reduced need for coronary artery bypass surgery, and reduction in ischaemic burden and mortality. In addition, when there is demonstrable ischaemia and viable myocardium in the CTO territory, there is further potential prognostic benefit from complete revascularisation. However, there has so far been a relative lack of randomised trial data to support the routine use of CTO-PCI. This paper reviews the current evidence surrounding this subject and discusses the arguments for and against CTO-PCI. It includes an exploration of the interventionalist's 'toolbox' and the techniques used in CTO-PCI, including a section on 'tips and tricks' for the most challenging cases. Finally, there is a discussion on the future of CTO-PCI including promising ongoing clinical trials and novel equipment that may improve outcomes and help to establish a more widespread adoption of CTO-PCI.

Keywords: Percutaneous coronary intervention (PCI), chronic total occlusion (CTO), drug-eluting stents (DES), retrograde, antegrade dissection/re-entry technique (ADR), microcatheter.

BACKGROUND AND HISTORICAL PERSPECTIVE

"If I had an enemy I would teach him angioplasty,"[1] were the words uttered by Andreas Gruentzig in 1980, 3 years after he performed the first percutaneous coronary intervention (PCI) in September 1977. Andreas Gruentzig would have witnessed the clinical benefits subside over time and, as the reality dawned that adverse events do occur post PCI, the initial honeymoon period and optimism of using PCI would have been diminished. Through further technological development, operator skill, and pharmacotherapy, PCI has now become the mainstream of treatment and the default choice for most revascularisation procedures. However, the last frontier of PCI that fills most operators with trepidation is chronic total occlusion (CTO)-PCI. There has been a large increase of innovations that have allowed the interventional cardiologist to treat diseases that were once only amenable to surgery.

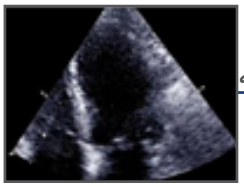
CHRONIC TOTAL OCCLUSIONS

PCI  VS  Medical Therapy

REDUCED ISCHAEMIC BURDEN
Reduction in ischaemic burden associated with reduction in death/MI in COURAGE nuclear substudy[7,17]

IMPROVED QOL AND SYMPTOMS
Observational studies show improvements in angina/dyspnoea symptoms, functional ability, and QOL, especially in symptomatic patients[23]

IMPROVED LVEF[43,44]



REDUCED NEED FOR CABG[47]

BETTER TOLERANCE OF FUTURE ACS
The presence of a non-IRA CTO has been shown to be an independent predictor of short and long-term mortality after STEMI[57]

HIBERNATING MYOCARDIUM CAN RECOVER AND REMODEL AFTER CTO-PCI
CTO-PCI had positive effects on LV remodelling in viable myocardium in CMR studies[43,44,57]

COMPLETE REVASCULARISATION
Observational data has found that mortality reduces with increasing completeness of revascularisation in those undergoing CTO-PCI[46]

IMPROVED SURVIVAL
Observational studies and meta-analyses suggests CTO revascularisation is associated with reduction in mortality[4,46]

LACK OF EVIDENCE
There is a lack of RCT or hard cardiovascular outcome data supporting use of CTO-PCI. Recent RCTs have had conflicting results[21,22]

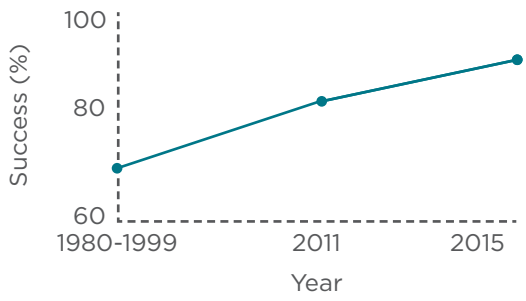
Many observational studies supporting CTO-PCI have compared successful vs. unsuccessful PCI, which is not equivalent to comparing with medical therapy[4]

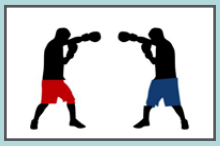
?

CTO-PCI IS A LONG PROCEDURE[51]
CTO procedures are longer than conventional PCI, and require more equipment and resources

CTO-PCI HAS HIGHER RISK
CTO-PCI has traditionally been associated with lower technical success and greater complication rates, including the risk of coronary perforation and cardiac tamponade[51]

However, procedural success is improving and complication rates are decreasing as operator experience increases, and with advanced techniques and equipment.[21,23,48,50]



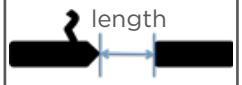


HIGHER RADIATION AND CONTRAST EXPOSURE[51]
CTO-PCI procedures involve increased fluoroscopy times, radiation doses, and contrast load

Figure 1: The boxers' guide to CTO-PCI: A selection of arguments for and against CTO-PCI.[4,8,44-54]

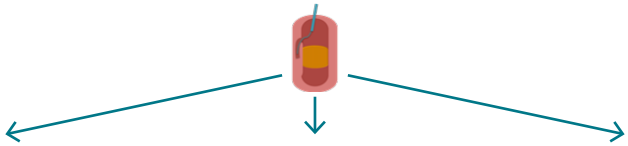
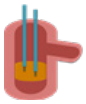
PCI: percutaneous coronary occlusion; MI: myocardial infarction; IRA: infarct related artery; CTO: chronic total occlusion; CABG: coronary artery bypass grafting; ACS: acute coronary syndrome; RCT: randomised controlled trial; LVEF: left ventricular ejection fraction; QOL: quality of life.

A) J-CTO SCORING SYSTEM

Entry shape of lesion		Calcification	Lesion bending >45°	Length of occlusion	Previous failed attempt
Tapered	Blunt				
					
Tapered=0 Blunt=1 Tapering at proximal cap scores 1		None=0 Present=1	No=0 Yes=1	<20 mm=0 ≥20 mm=1	No=0 Yes=1

0=Easy	1=Intermediate	2=Difficult	≥3=Very difficult

B) ANTEGRADE TECHNIQUES

CONVENTIONAL		ANTEGRADE DISSECTION AND RE-ENTRY		
Single wire Over-the-wire microcatheter Single wire penetrates lesion using wire escalation with controlled drilling and penetration/step up-step down wire approach 	**Side branch anchor** Wire anchored in proximal side-branch +/- balloon to increase guide catheter support 	Intentional subintimal dissection using loop of wire tip or blunt micro-dissection catheter 		
Parallel wires  First wire anchored in subintimal space, acts as marker A second stiffer wire advanced into true lumen via micro-catheter		STAR  Advance wire in subintimal space until spontaneous re-entry of wire to true lumen	MiniSTAR/LAST  Wire re-entry as early as possible after occlusion using loop of wire tip (MiniSTAR) or specialised guide wire with distal bend (LAST)	Stingray  Uses specialised Stingray balloon in subintimal space, with two exit ports, one directed towards true lumen for wire to pass through
Seesaw wires  Uses two wires, each with an over the wire microcatheter				

C) RETROGRADE TECHNIQUES

Use both antegrade and retrograde (via collaterals) wire with aim of connecting passages made by each wire

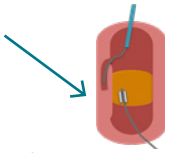
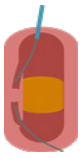
Kissing wire cross  Both wires are in the true lumen and are each gradually advanced through lesion until they overlap	**Antegrade balloon dilatation (1)**  Similar to Kissing wire cross but uses balloon to dilate and expand antegrade channel towards retrograde channel	**CART**  Antegrade wire is in subintimal space Retrograde ballooning to advance and connect passages
Intentional antegrade dissection  Antegrade penetration of subintimal space and balloon inflation until connection to retrograde channel	**Antegrade balloon dilatation (2)**  Antegrade wire in true lumen, retrograde wire in subintimal space Antegrade ballooning to disrupt body of occlusion and connect channels	**Reverse CART**  Both wires are in the sub-intimal space Progressive advancement of antegrade wire to connect channels

Figure 2: A) J-CTO score is calculated by addition of individual scores, allowing lesions to be categorised as easy, intermediate, difficult, or very difficult; B) and C) Antegrade and retrograde techniques in CTO-PCI.[52,53] CTO: chronic total occlusion; PCI: percuateous coronary intervention; CART: controlled antegrade and retrograde tracking.

Adapted from Morino et al.[24]

CTOs are a subset of coronary artery disease (CAD) defined as coronary arteries with absent anterograde flow over >12 weeks duration.[2] They have been found in ≤20% of coronary disease cases diagnosed at angiography.[3] The technical difficulty associated with treatment of CTOs has led to the conclusion that it is the 'final frontier' in interventional cardiology.[3-5] The low intervention rates are commonly due to the misconception that intervening on a CTO will have limited benefit, with higher complication rates. CTO-PCI is no enemy, but a friend, and we present here the evidence, treatment strategies, and indications that allied to newer devices and techniques, ensure safe and effective treatment of these complex lesions, and often forgotten patients.

JUSTIFICATION

Werner et al.[6] demonstrated that regardless of the degree of collateralisation, the area of myocardium distal to the CTO lesion is always ischaemic. Ischaemia is effectively relieved by a successful CTO-PCI, although success depends on the vessel intervened upon.[7,8] Additionally, complete, as opposed to incomplete, revascularisation has mortality benefits regardless of whether surgical or percutaneous revascularisation is used, supporting the notion that CTO-PCI reduces ischaemic myocardial burden (Figure 1).[2]

Intervention has been linked to a lower incidence of ventricular arrhythmias,[9] improved left ventricular (LV) function,[10] and improvements in quality of life (QoL).[11] A 2017 study by Sotomi et al.[12] examined the effects of CTO-PCI on electrical stability and LV function in a single-centre, prospective, observational study. It evaluated electrical stability as a surrogate marker for ventricular arrhythmias, using signal-averaged electrocardiogram (SAECG), and assessed systolic and diastolic function using speckle tracking echocardiography. They found no improvements in electrical stability following CTO-PCI, but did find some evidence of enhancements in systolic and diastolic function.

ISCHAEMIA

Clinically, the primary driver towards CTO-PCI is symptomatic angina with associated coronary ischaemia. The American cardiovascular societies' appropriate use criteria (AUC) outlines 180 clinical scenarios aiming to guide clinicians in making decisions about intervention in patients with CAD including CTOs.[13] The AUC heavily emphasise ischaemic burden as a reason for revascularisation when there is a large area of ischaemia, even in the absence of symptoms. The 2014 European revascularisation guidelines recommend that CTO-PCI be considered when there is an expected reduction in myocardial ischaemia (Class IIa, Level of Evidence B).[14,15] CTO-PCI has been shown to significantly decrease the ischaemic burden from 13.1% to 6.9%. Moreover, mortality has been shown to increase in several studies if the percentage of myocardium at risk is >7–10%.[16,17]

SYMPTOMS

A key objective of CTO-PCI is to induce relief of symptoms, improve exercise capacity, and improve QoL. Anecdotally there is a tendency to underestimate the symptoms attributable to CTOs. This is because there tends to be a higher prevalence of angina chest pain rather than shortness of breath.[2] Large observational studies looking at the impact of CTO revascularisation have found improvements in patient's angina and QoL (assessed by QoL indexes).[18-20] The beneficial effects include improved physical activity ($p=0.01$), rarer anginal episodes ($p<0.001$), and greater treatment satisfaction ($p=0.03$) compared with failed interventions.[20]

Considering the importance of symptom control on the benefit of CTO intervention, few studies exist that compare its efficacy with optimal medical treatment (OMT). DECISION CTO[21] is a randomised controlled trial (RCT) which began recruitment in 2010 and presented its findings at the American College of Cardiology (ACC) scientific sessions in March 2017. It recruited 834 patients and was due to follow-up for a total of 5 years; however, due to problems with recruitment it stopped at 3 years. The CTO-PCI success rate was 91.1%. At 3 years, the combined primary endpoint of all-cause death, myocardial infarction (MI), stroke, and repeat revascularisation in the intention-to-treat population was similar for patients on OMT and CTO-PCI (19.6% versus 20.6%; $p=0.008$ for non-inferiority). Furthermore, there were no differences in angina or QoL scores at 1-year.

This study contrasts that of the EUROCTO[22] club trial which began recruiting in 2012 and presented its 12-month findings in May 2017 at the EuroPCR congress. It was a multicentre RCT, which examined the symptomatic benefit of CTO-PCI versus OMT (aspirin, statin, angiotensin-converting-enzyme [ACE] inhibitor, and two anti-anginals at the

maximum tolerated dose). The study recruited 407 patients from 26 different countries. Using the Seattle Angina Questionnaire (SAQ) it showed a benefit of using CTO-PCI versus OMT (p=0.009) and reported improvements in the Canadian Cardiovascular Society (CCS) angina score (p<0.001). It also showed that improvements in QoL, angina stability, physical activity, and treatment satisfaction were numerically higher in CTO-PCI compared with OMT. There were comparable MACCE rates at 12 months.[23]

DECISION-CTO and EUROCTO highlighted the continued controversy about trial data which has led to a divergence in the real-world management of CTOs across different institutions and countries.

CLASSIFICATION AND SCORING SYSTEMS

Scoring systems help to predict the probability of procedural success. The 2011 Japanese CTO (J-CTO) is based on a multicentre retrospective analysis of nearly 500 CTO procedures. It identified five independent predictors of the ability of the guidewire to cross the lesion within the first 30 minutes.[24] It applies one point for each of these variables when present: i) calcification; ii) bending >45° in the CTO segment; iii) a blunt proximal cap; iv) when the length of the occluded segment is >20 mm; and v) a previously failed attempt. The CTO case complexity was further stratified into easy (J-CTO score=0), intermediate (J-CTO score=1), difficult (J-CTO score=2), and very difficult (J-CTO score=3–5) (Figure 2).

The J-CTO study had limitations, including the fact that the overall success rate was 72% versus the current standard of 85–90%,[24,25] and the under-representation of retrograde PCIs. Alessandrino et al.[26] proposed the Clinical and Lesion (CL) related score. This emerged from a single-centre prospective trial of 1,657 patients. It includes variables such as the anatomy of the proximal cap, grade of lesion calcification, left anterior descending (LAD) or non-LAD CTO, lesion length >20 mm, and history of coronary artery bypass grafting or MI. All variables are then adjusted on the basis of an odds ratio.

ORA,[27] PROGRESS-CTO,[28] and RECHARGE[29] are more contemporary scoring systems, taking into account the hybrid approach. The ORA scoring system uses ostial location, Rentrop Grade <2 (Grade 0 has no angiographic evidence of filling and 4 is fully filled), and age ≥75 years; with these measurements it is possible to assign each lesion a technical difficulty and therefore predict procedural success.[27] The PROGRESS-CTO score also uses four variables (proximal cap ambiguity, absence of retrograde collaterals, moderate or severe tortuosity, or a circumflex CTO). This was developed from the PROGRESS registry and was validated against the J-CTO scoring system. The RECHARGE[29] score uses six variables (blunt stump, calcification, tortuosity >45°, lesion length >20 mm, diseased distal landing zone, and previous CTO vessel bypass graft) and was validated against the PROGRESS and J-CTO scoring systems.

Yu et al.[30] have developed the Korean Multicentre CTO CT Registry (KCCT) score in 2017. Unlike the aforementioned scores, it uses computed tomography (CT) angiographic imaging to predict the difficulty of navigating the CTO within 30 minutes as well as procedural success. It analysed 684 CTO lesions with CT and identified proximal blunt entry, proximal side branch, bending, occlusion length >15 mm, severe calcification, whole luminal calcification, reattempt and >12 months or unknown duration of CTO as being independent predictors of success. A KCCT score <4 predicts the ability to cross the lesion within 30 minutes (p<0.05).

'THE KIT'

Vascular Access

The femoral approach allows larger and more supportive guiding catheters; however, radial access increases patient comfort and reduces the incidence of vascular complications. With complex lesions (J-CTO >3) we recommend long 8 Fr and 7 Fr femoral sheaths. The Arrow® long armoured-style sheath is particularly useful for this and also has great trackability in patients with peripheral vascular disease. This will ensure a hybrid approach is feasible, if necessary, and will also allow the insertion of intravascular ultrasound (IVUS), microcatheters, guideliners, anchor balloons, and multiple wires and balloons if required.

Guide Catheters

Guide catheter selection is extremely important; if the procedure requires additional support to prevent prolapse then a larger diameter catheter should be used (AL1 for the right coronary artery, and EBU for the left coronary artery, usually with preference to size up [e.g. use EBU4 in preference to EBU3.5]). Softer tip catheters are emerging such

as Hyperion™ (Asahi Intecc, Aichi, Japan), which may cause less trauma to the ostia and are marginally more steerable. In general, two guide catheters are used for each CTO-PCI case to allow the operator to seamlessly switch between antegrade and retrograde approaches. A mandatory dual coronary injection is performed to assess: i) a clear understanding of the location of the proximal cap; ii) occlusion length; iii) the presence of side branches and size and quality of the distal vessel; and iv) the presence of collaterals suitable for retrograde technique.

Wires and Microcatheters

Failed CTO-PCI is frequently caused by the inability to cross the CTO with a guidewire. There are three separate steps required to cross a CTO: i) penetrating the proximal fibrous cap; ii) traversing the body of the CTO to reach the distal fibrous cap; and iii) penetrating the distal fibrous cap. Dedicated guidewires for crossing CTOs have been developed. They are broadly divided into two groups:

- Hydrophilic (polymer coated) wires (e.g. Fielder™, Fielder XT [Asahi], Pilot 200® [Abbott, Illinois, USA]) – offer manoeuvrability in tortuous vessels and passage through micro-channels into the true lumen. They can, however, increase the incidence of sub-intimal perforation.
- Non-hydrophilic (non-polymer coated) wires (e.g. Miracle Bros® 3-12 [Asahi], Confianza Pro [Asahi]), are typically more controllable, provide better tactile feel, and are less likely to cause vessel dissection. They can be used to cross the fibrous cap as they have greater penetration force. They are graded in grams according to the penetration force they can withstand in grams during bench testing. This does give an idea of penetrability *ex vivo,* but operators should be cautious that, in regard to *in vivo* with microcatheter support, these values are only a guide and can be higher if used with anchor balloons or differing microcatheters. There are also newer steerable intra-occlusion wires such as the Gaia family (Asahi) (first, second, and third). The unique property of these wires is their ability to steer the wire with the use of a microcatheter within the occlusion.

It is possible to cross the CTO with a guidewire alone, although in the majority of J-CTO >2 cases a microcatheter for support is considered mandatory (e.g. Corsair [Asahi], FineCross® [Terumo, Leuven, Belgium], M-CATH [Accrostak, Geneva, Switzerland], NHancer [IMDS, Roden, Netherlands], Turnpike [Vascular Solutions, Minnesota, USA]). This allows the operator to provide increased wire force to the proximal fibrous cap. An alternative is an over-the-wire (OTW) balloon catheter (e.g. 1.25–1.5 balloon diameter), which, allied to increase force, allows pre-dilation once the CTO is crossed. OTW has more or less disappeared from use in CTO-PCI work and is essentially historic because the main limitation is the inability of OTW to track through complex CTOs.

'TIPS AND TRICKS' AND CHRONIC TOTAL OCCLUSION TECHNIQUES

The Stingray Balloon and Wire

When unable to penetrate the fibrous cap, a dissection flap can be created to circumnavigate the occlusion. The Stingray™ LP balloon (Boston Scientific, Massachusetts, USA) is a flat balloon that can be inflated in an intended dissection plane. There are two radiopaque markers on the Stingray balloon and an exit hole just proximal to each marker on the opposite face of the balloon. One exit port will lead the wire into the adventitial side of the artery distal to the occlusion and the other to the luminal side, therefore allowing re-entry and bypass of the CTO. The Stingray Wire (Boston) is a stiff wire with a pre-shaped 28° tip and a barb on the end to facilitate re-entry. It can be carefully advanced in the Stingray balloon and directed towards either the adventitia or luminal layer.

Anterograde

Anterograde is the most common approach to CTO-PCI. Progressively stiffer hydrophilic/non-hydrophilic wires are used sequentially until the proximal cap is penetrated or the wire advanced within the lesion. Failure after initial wire entry in the lesion has led to specialised techniques for recanalisation of CTOs. The antegrade dissection/re-entry technique (ADR) and subintimal tracking and re-entry (STAR) techniques (Figure 2) were pioneered by the work of Colombo et al.[31] and Carlino et al.[32] The STAR technique is a less well-controlled method of performing an ADR, where a hydrophilic wire in the shape of an 'umbrella handle' is knuckled though the intimal dissection planes until it enters the distal true lumen. ADR with the use of the aforementioned Stingray balloon and Crossboss™ (Boston) device allow the operator to perform a more precise and limited dissection than what was previously observed with the STAR

technique. These techniques have been essential in the remarkable improvements in the procedural success rates.

Retrograde

A retrograde approach can be broadly described as any approach that uses donor collaterals to deliver interventional equipment to facilitate the opening of a CTO. Unlike early procedures, this procedure has evolved to use septals (which have a straighter course) or the more tortuous epicardial vessels as the common conduit arteries.[33] The goal of the retrograde approach is to target the distal cap, which could be softer than the proximal one. It should be noted that the retrograde approach should only be performed by experienced operators who have mastered the anterograde approach. For all CTO-PCI, the activated clotting time (ACT) should be measured throughout ensuring an ACT >300, and further heparin introduced if required.

Various techniques have been described for retrograde CTO-PCI. These include passing the guidewire retrogradely through the distal cap within the true lumen with balloon dilatation before antegrade guidewire passage and PCI. Alternatively, passing the wire into the subintimal space and enlargement with sequential balloon dilatations before connecting with the true lumen retrogradely (controlled antegrade and retrograde tracking [CART] technique). If the subintimal space is enlarged from the anterograde then it is called the reverse CART technique (Figure 2). If the retrograde wire and microcatheter is eventually inserted into the antegrade guide then the RG3® (Asahi) wire is utilised to pass through the occlusion and is externalised in the opposite sheath. Conventional PCI can then be performed on the RG3 wire in an antegrade manner. It must be emphasised that at no time should contrast injections be performed in the antegrade guide prior to stenting, as this will only facilitate dissection planes and can cause large haematomas that could lead to compression of cavity and haemodynamic compromise.

The Hybrid Approach

The hybrid approach was proposed in 2012 and has been shown to be effective in ≤90% of cases with fewer complications (cardiac tamponade: 0.4%, periprocedural MI: 1.0%, death: 0.4%).[9,34] This is effectively a combination of the anterograde and retrograde techniques described above.

Future Directions in Chronic Total Occlusion-Percutaneous Coronary Intervention

Two further ongoing clinical trials should further clarify the benefits of CTO-PCI. OPEN-CTO[35] is a prospective observational registry of patients enrolled in North America utilising the hybrid approach. It aims to assess the safety, health and QoL, and cost effectiveness of the method. Initial results have been promising (Table 1).

Table 1: A summary of a selection of important studies relating to CTO-PCI.

Study	N	Design	Primary outcome	Key findings	Author comments
Shaw et al.[49]	314	Nuclear substudy of the COURAGE trial Compared PCI + MT vs. MT only in patients with stable CAD	≥5% reduction in ischaemic burden	PCI+MT resulted in greater reduction in ischaemic myocardium vs. MT alone Patients with moderate-to-severe ischaemic burden (≥10%) had even greater reductions with PCI+OMT (78% vs. 52%; p=0.007) Those with ≥5% ischaemia reduction had lower unadjusted risk of death/MI (p=0.037), especially if ≥10% ischaemic burden (p=0.001) Study recommended treatment target of ≥5% ischaemia reduction	This study emphasised the importance of ischaemic burden, and the effect of reduction on outcomes
Safley et al.[8]	301	Retrospective study of myocardial perfusion imaging in those undergoing CTO-PCI	Reduction in ischaemic burden	53.5% had significant (≥5%) reduction in ischaemic burden Average ischaemic burden reduced from 13.1% to 6.9% (p<0.001) Patients with ≥12.5% ischaemic burden most likely to benefit	Large study confirming beneficial effects on ischaemia reduction

Table 1 continued.

Study	N	Design	Primary outcome	Key findings	Author comments
Ladwiniec et al.[54]	34	Prospective study assessing effect of CTO-PCI on FFR in donor vessel (of collaterals)	FFR in donor vessel	Recanalisation of a CTO was associated with an improvement in donor artery FFR (0.782–0.810; p=0.001) Greater changes seen where donor vessel FFR ≤0.8	Even patients with well-collateralised CTOs may benefit from PCI
Dzavik et al.[55]	381	RCT of AMI patients with persistently occluded IRA-PCI vs. MT only	Vessel patency and LVEF	PCI associated with greater vessel patency at 1 year vs. MT (83% vs. 25%; p<0.001) but no difference in LVEF, LVESVI, or LVEDVI between groups	Very selected population that is not representative of many CTO-PCI candidates. Limited viability assessment
Grantham; Saint Luke's Health System[35]	1,000	Ongoing prospective multicentre observational registry assessing outcomes after CTO-PCI using the hybrid approach	Safety, health status outcomes, cost	6-month results showed that PCI was associated with improved quality of life, anginal stability, treatment satisfaction, and reduced angina frequency, physical limitation, SOB, and depression High technical success (89%) and low rate of complications	Large study showing significant QoL benefits. Due to complete in Dec 2017
Henriques et al.[56]	304	RCT of STEMI patients undergoing PPCI with concurrent CTOs, randomised to early PCI of CTO or MT	LVEF/LVEDV on CMR at 4 months	No significant difference in 4-month MACE (5.4% vs. 2.6%; p=0.25) Patients with CTOs in LAD had significantly higher LVEF with PCI vs. MT (47.2 vs. 40.4%; p=0.02). In other patients, there was no significant difference in LV function/volume Low numbers of adverse events, additional CTO-PCI <7 days following PPCI felt to be safe CTO-PCI procedural success was 73%	4 months perhaps not long enough to demonstrate significant benefit. Low success rates in PCI arm of trial compared to average experience
Lee et al.[47]	1,173	Registry study comparing successful vs. unsuccessful PCI	Mortality, need for CABG	Successful CTO-PCI not associated with reduction in all-cause mortality but significant reduction in need for CABG (HR: 0.02, CI: 0.006-0.06; p<0.001), and TVR (HR: 0.15, CI: 0.1-0.25; p<0.001)	High success rate and therefore small size of unsuccessful PCI group
George et al.[46]	13,443	Long-term follow-up of patients on UK Central Cardiac Audit Database undergoing elective CTO-PCI Compared successful vs. unsuccessful PCI and CR vs. IR	Cardiovascular outcomes and mortality	Successful revascularisation of at least one CTO associated with reduction in long-term mortality (HR: 0.72, CI: 0.62–0.83; p<0.001) Trend towards reduced mortality with increasing completeness of revascularisation (p<0.001) CR associated with reduction in mortality vs. IR (HR: 0.7; CI: 0.56–0.87; p=0.002)	Large study with long-term follow-up data. No data on lesion complexity or details of medical therapy were available
Galassi et al.[45]	1,914	In-hospital outcomes from the ERCTO registry (16 centres)	Success, technical information, and cardiovascular outcomes	CTO-PCI procedural success was 82.9%. Antegrade approach more successful compared to Retrograde approach (83.2% vs. 64.5%; p<0.001) Retrograde approach also associated with higher rates of coronary perforation, procedural and fluoroscopy times and contrast load Similar rates of 30-day MACE regardless of approach. Low rates of overall complications (CIN: 0.9%, stroke: 0.05%, death: 0.3%) that are comparable to non-CTO-PCI	Higher complications with retrograde approach may be partially explained by reduced experience with newer techniques
Sapontis et al.[48]	380	Retrospective study of patients undergoing Hybrid Approach CTO-PCI. Compared successful vs. unsuccessful PCI	Factors associated with failure	CTO-PCI procedural success was 91.3%. Lesions in failed CTO-PCI were longer, more tortuous, had more proximal cap ambiguity and blunt stumps, higher mean J-CTO scores, and were less likely to have collaterals amenable for retrograde approach	Findings demonstrate utility of J-CTO score in planning CTO-PCI

Table 1 continued.

Study	N	Design	Primary outcome	Key findings	Author comments
Pancholy et al.[4]	-	Meta-analysis of 13 studies comparing successful vs. unsuccessful CTO-PCI	All-cause mortality (short-term: n=3,932; long-term: n=6,403)	Successful CTO-PCI was associated with significant reduction in short-term (OR: 0.218, CI: 0.095–0.498; p<0.001) and long-term mortality (OR: 0.391; CI: 0.311–0.493; p<0.001)	Only included studies with high proportion of stent use. Low heterogeneity
Park et al.[21]	834	RCT of CTO-PCI vs. OMT	MACCE	Successful CTO-PCI was not associated with a reduction in MACCE at 3 years. There were no differences in angina or QoL at 1 year	Trial was stopped early due to poor recruitment
Werner et al.[22]	407	RCT of CTO-PCI vs. OMT	Symptom control	Successful CTO-PCI was associated with improved symptom control at 1 year There were numerical improvements in angina, physical activity, and treatment satisfaction. MACCE rates were comparable	The long-term safety evaluation is awaited. The trial didn't recruit the pre-planned number of patients

AMI: acute myocardial infarction; CAD: coronary artery disease; CI: confidence interval; CIN: contrast-induced nephropathy; CR: complete revascularisation, CTO: chronic total occlusion; ERCTO: European Registry of Chronic Total Occlusion; FFR: fractional flow reserve; IR: incomplete revascularisation; LVEDV: left ventricular end diastolic volume; LVEDVI: left ventricular end-diastolic volume index; LVEF: left ventricular ejection fraction; LVESVI: left ventricular end-systolic volume index; MACE: major adverse cardiovascular events; MT: medical therapy; n: number of patients; PCI: percutaneous coronary intervention; QoL: quality of life; RCT: randomised controlled trial; TVR: target vessel revascularisation; OMT: optimal medical therapy; MI: myocardial infarction; J-CTO: Japanese CTO; LAD: left anterior descending; HR: hazard ratio; OR: odds ratio; MACCE: major adverse cardiovascular and cerebrovascular event.

SHINE-CTO[36] is a USA single-centre RCT of CTOs to PCI versus sham procedure, and will assess the impact on QoL.

TECHNOLOGICAL DEVELOPMENTS AND THE SPECIFICS TO CHRONIC TOTAL OCCLUSION-PERCUTANEOUS CORONARY INTERVENTION

Technological developments may provide the 'knockout blow' for CTO-PCI. Some promising developments are described below.

Orbital Arthrectomy

Orbital atherectomy can facilitate plaque removal and softening, improve lesion compliance, and aid dilatation, especially in severely calcified lesions.[37] Though underused, it has been shown to be effective at facilitating angioplasty in resistant CTOs where device crossing is initially unsuccessful.[38]

Drug-eluting Balloons

Drug-eluting balloons have been the target of research looking at lowering the rates of in-stent restenosis. They also require a shorter duration of antiplatelet therapy. Koln et al.[39] investigated the feasibility and safety of its use in CTOs. They looked at 66 cases of anterograde CTO intervention (retrograde was not investigated due to the high prevalence of dissection). They found that restenosis (11.8%) and reocclusion (5.9%) rates were comparable to CTO-PCI. This was a small, non-randomised study and the lesions themselves had to have had good predilation results, which biased their selection. In view of its potential, further RCT evidence is required.

SoundBite

The SoundBite Crossing system[40] (SoundBite Medical Solutions, Inc, Quebec, Canada) is a novel device using shockwaves (short-duration, high amplitude pressure pulses) to facilitate crossing the proximal cap. It propagates shockwaves to its tip, using it as a micro 'jackhammer' to penetrate the lesion, and has recently undergone an *ex vivo* trial in an amputated leg. Further trials *in vivo* are expected. Although it is currently being used in peripheral vascular disease, it has potential for use in coronary disease.

Collagen and Collagenase

A collagen-rich matrix is present in CTO lesions and forms a barrier at the proximal cap. Collagenase is

an enzyme that degrades Type 1 collagen, and may facilitate lesion crossing. Phase I studies have shown it to be a feasible and safe therapy.[41]

CONCLUSIONS

CTO's have a significant impact on health and mortality, yet are undertreated. CTO-PCI offers potential benefits and should be considered for patients with symptoms of, or demonstrated, ischaemia, and viable myocardium in the CTO territory. Modern techniques and scoring sytems allow operators to successfully tackle complex and challenging CTOs. PCI success rates and safety are improving with contemporary trials providing evidence to challenge common misconceptions and allow patients, who may traditionally have been left untreated, to gain the potential benefits from CTO-PCI.

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FETAL PERICARDIOCENTESIS

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ABSTRACT

Fetal pericardiocentesis is a safe and effective procedure that is used to drain pericardial effusion in selected fetuses. The aim of the procedure is to reduce the risk of pulmonary hypoplasia, the development of cardiac tamponade and fetal hydrops, and in some cases to allow fetal lung maturity, improving fetal extraction with a better haemodynamic and respiratory condition. In this review, we discuss the indications, technical procedure, and the outcomes of the fetal pericardiocentesis reported in the literature.

Keywords: Pericardiocentesis, pericardial effusion, cardiac tumours, pulmonary hypoplasia.

INTRODUCTION

The pericardium is a double-walled fibroelastic sac containing the heart and origin of the great vessels. It consists of two layers: an outer or parietal layer and an inner or visceral layer. The pericardium prevents sudden dilation of the heart, especially the right side, and heart and great vessel displacement; it minimises heart friction with the surrounding structures and prevents the spread of infection or cancer cells from the lung and pleura. In between the two layers there is a small amount of fluid, a plasma ultrafiltrate produced by the visceral layer. This liquid facilitates friction-free movement of the heart during systole and diastole within the pericardial sac. During ventricular systole, the pericardial space around the ventricles expands and the pericardial fluid moves into this expanding space, and during ventricular diastole, the pericardial space around the ventricles disappears as the pericardial fluid moves away from the field of view. This phasic movement is characteristically demonstrated by a colour Doppler signal within the pericardial space opposite to the direction of the colour Doppler signal seen within the ventricles.[1]

PERICARDIAL EFFUSION

Definition

The sonographic finding of fluid with an amount of <2 mm is frequent during ultrasound examination and is seen in >40–50% of normal fetuses, especially when the ultrasound beams are perpendicular to the ventricular walls. This finding has no clinical significance and is not considered to be pericardial effusion.[1-4]

Pericardial effusion is defined on ultrasound examination when the heart is partially or completely surrounded by liquid that is seen in all projections, is usually present around the atrioventricular groove, and the larger region is >2 mm[5,6] and surpasses the atrioventricular union. If it measures <4 mm it is considered small and if it measures >4 mm it is considered large.[7] In cases of massive pericardial effusion, a posterior displacement of the lungs can be found on the four-chamber view (Figure 1). The main differential diagnosis is pleural effusion, which appears on sonography, a fluid layer surrounding the lungs.

Aetiopathogenesis

Pericardial effusion can be found isolated or associated with different abnormalities described

in the literature (Table 1). The incidence is about 0.64–2.00%.[6,8] It is necessary to perform a comprehensive fetal study ultrasound to rule out the different causes to which it is associated. In most cases the effusion occurs as a manifestation of fetal hydrops (immune or non-immune), which is necessary to discard.[5] The mechanisms for development of pericardial effusion can be transudative (obstruction of lymphatic drainage, increased lymphatic pressure) or exudative (secondary to inflammation, infection, malignancy, or autoimmune). The pathogenesis depends on the cause.

A complete sonographic evaluation of the fetus and maternal blood tests must be performed to determine the presence of abnormal group antibodies, rule out viral infections, fetomaternal haemorrhage, and in some cases amniocentesis for karyotyping and umbilical blood sampling to confirm the presence of anaemia and viral infections. If no causes are found the pericardial effusion is transient and idiopathic, with a better prognosis, and almost 45% of the cases will resolve spontaneously.[8]

For cases of high-output cardiac failure secondary to fetal anaemia, arrhythmias, or congenital heart disease, studies should be performed to make the differential diagnosis. In cases of fetal anaemia, pericardial effusion is an early sign of hydrops, or appears in a hydropic fetus, which should be treated to resolve the fetal condition.

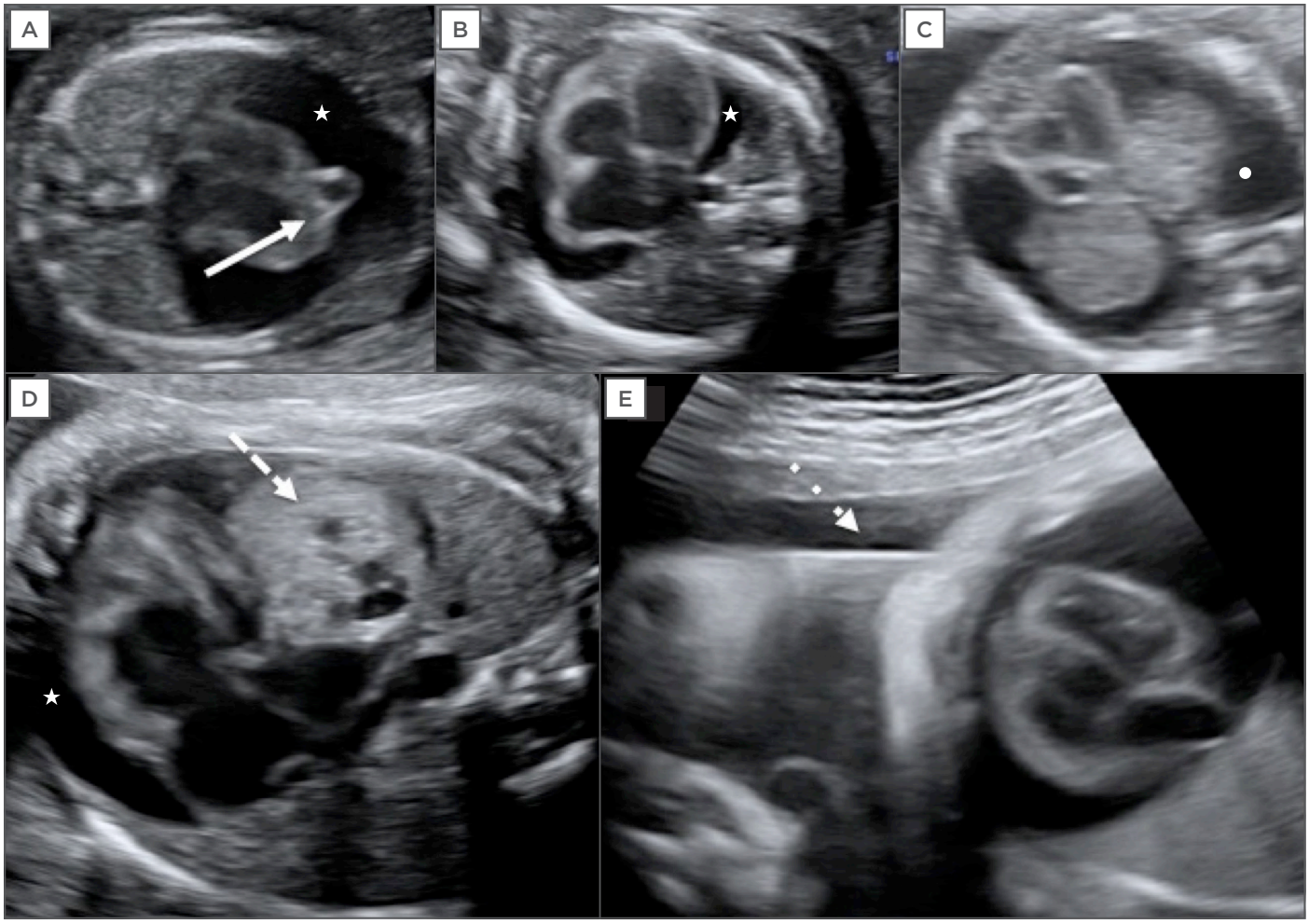

Figure 1: Fetal echocardiography.
A) Large pericardial effusion (white star) associated with fetal cardiac diverticulum at 15 weeks of gestation (continuous arrow); B) Large pericardial effusion (white star) associated with fetal anaemia; C) Differential diagnosis with pleural effusion showing the fluid around the lungs (white dot); D) Large pericardial effusion (white star) associated with cardiac teratoma at 32 weeks of gestation (discontinuous arrow); E) Pericardiocentesis in this fetus at 32 weeks, the needle crosses the fetal thorax (dotted line arrow).

Table 1: Causes of pericardial effusion.

Cardiac abnormalities Cardiac malformation Arrhythmia Cardiac tumours: teratoma, rhabdomyoma
Hydrops Immune Non-immune Cardiac abnormalities Infections: parvovirus B19, cytomegalovirus Anaemia Arrhythmia Cardiomyopathy Miscellaneous: mediastinal teratoma, lymphohistiocytosis Placental chorioangioma
Infections Cytomegalovirus, parvovirus B19
Anaemia
Intrauterine growth restriction
Aneuploidy
Placental haemorrhage
Cerebral abnormalities Intracranial haemorrhage Subdural haematoma
Thorax abnormalities Congenital cystic adenomatoid malformation Congenital diaphragmatic hernia
Vascular abnormalities Haemangioma Hepatic haemangioendothelioma Portohepatic shunt Kasabach–Merritt syndrome
Abdominal wall malformations Omphalocoele Pentalogy of Cantrell
Genitourinary malformation Polycystic kidney disease Multicystic dysplastic kidney Posterior urethral valve syndrome Hypospadias
Spinal malformation Sacrococcygeal teratoma
Transient pericardial effusions

Adapted from Kyeong et al.[8]

In the case of cardiac teratomas, the pericardial effusion is probably due to the irritative stimulus of the tumour on the pericardial layers and rupture of the cystic areas in the pericardium caused by the multicystic nature of pericardial teratomas. Furthermore, the tumour can cause mechanical obstruction of the venous return and the thoracic duct which interferes with the lymphatic drainage, leading to the development of pericardial, pleural effusions, ascites, and fetal hydrops. Oesophageal compression can cause polyhydramnios. Pericardial effusion and mass effect are responsible for fetal pericardial tamponade.[9]

Pericardial effusion is rarely associated with another cardiac tumour. Some cases of cardiac rhabdomyomas with pericardial effusion have been described in literature due to the rapidly growing mass and the development of cardiac arrhythmia. There are few cases associated with fibromas and cardiac haemangiomas.[8,10,11] In cases of pericardial effusion associated with cardiac diverticula, it is believed to be caused by the rubbing of the diverticulum with the pericardium walls.

PERICARDIOCENTESIS

Definition

Fetal pericardiocentesis is the removal of fluid with a percutaneous catheter or needle from the pericardial sac. It is a safe and effective method to drain the effusion. It must be performed after 16 weeks of gestational age to avoid complications, just as with amniocentesis.

Indications

The aim of pericardiocentesis is to decompress the fetal chest, allowing the expansion of the lungs, reducing the pressure in the fetal venous system, causing a theoretical risk reduction for pulmonary hypoplasia and fetal hydrops.

Depending on the aetiology, for some cases a single procedure may resolve the condition. There have been reports in the literature of spontaneous resolution especially in idiopathic cases, but also in cases associated with cardiac diverticula.[5,8,12,13] Since pericardiocentesis is considered a technique with a low risk to the fetus, it should be the treatment of choice in selected cases, such as large pericardial effusions that do not disappear or increase in a short period of time, to avoid the risk of pulmonary hypoplasia with severe sequelae to the fetus.[12] For fetuses with moderate pericardial effusions outpatient follow-up should be done according to the underlying cause and fetal status, which may require weekly controls or controls every 48 hours.

In isolated pericardial effusion, the persistence of pericardial effusion is more important than the size. In some studies there were no correlations between the size of the pericardial effusion

and the regression of pericardial fluid, adverse outcomes, or mortality rate.[5,6,8] The pericardial effusion associated with cardiac tumours, usually teratomas, benefits from performing an intrauterine pericardiocentesis.[9,14-16] The decompression of the pericardial cavity reduces the risk that the fetus may develop non-immune fetal hydrops. In these cases, intrauterine pericardiocentesis can help to reduce fetal hydrops with prolongation of pregnancy until lung maturity is reached, which prevents or delays the development of cardiac tamponade or pericardial effusion relapse. The use of pericardiocentesis immediately prior to delivery improves ventilation and haemodynamic stability in the preterm infant, prevents severe cardiorespiratory distress, facilitates neonatal stabilisation and intubation, and allows planned rather than emergency cardiothoracic surgery.[15-18]

It has been proposed that fetuses with cardiac tumours such as teratomas must be followed to assess the presence of fetal hydrops. In cases where hydrops develops and the fetus is >28–30 weeks and matured, delivery and postnatal intervention could be considered. In cases where there is insufficient lung maturity, pericardiocentesis can be performed to delay delivery for 1–2 weeks and allow time to mature the fetus before delivery. For fetuses <28 weeks old with severe pericardial effusion it is recommended to perform pericardiocentesis and if the effusion recurs, it is sometimes necessary to perform this procedure again[16,19] and serial pericardiocentesis may be required to avoid prematurity with its related complications.[12,20-23] Therefore, the aetiology associated with the pericardial effusion, gestational age, and fetal status should be taken into consideration to assess the need for fetal lung maturation with corticosteroids. We do not consider that fetal lung maturation is required in all cases <34 weeks of gestational age just because the procedure is being performed, but it should be considered in those cases where fetal status is compromised and could require an immediate delivery if fetal deterioration occurred. There have been reported cases in which a pericardial-amniotic shunt was placed and, in some cases, associated with pericardiocentesis. However, there are limited data available and further evaluation of its efficacy is needed.[12,24]

Technique

Fetal pericardiocentesis should be performed under sonographic guidance as an outpatient procedure. Two people are needed for the procedure: an operator to control the needle and transducer, and an assistant to help in aspiration of the fluid.

A fetal ultrasound should be performed initially to locate the most suitable place for puncture. The mother should be positioned as horizontally as possible, to allow better access to the amniotic cavity. Skin preparation with antiseptic solution and sterile field placement should be performed. The ultrasound probe should be prepared with an antiseptic solution prior to each procedure, since the probe is not covered with a sterile drape. We use a multi-frequency convex transducer with a 60x13 mm footprint and a 2–7 MHz bandwidth.

With a short 22-gauge needle, local anaesthesia is administered at the puncture site and with the local anaesthetic needle, a preliminary exploration to confirm the direction of the needle approach is made. Then, under direct visualisation a 20–22-gauge needle should be used to access the fetal chest. We recommend using a 20-gauge needle just as when performing amniocentesis. The puncture needle is gently inserted in the maternal skin and advanced through the uterus, amniotic cavity, and fetal chest under echographic guidance. Then, with a syringe, the fluid should be drained and finally the needle removed under echographic visualisation. The complete procedure must be performed with ultrasound guidance with continuous visualisation of the needle. Follow-up is performed every 48 hours during the first week, and after that the follow-up interval will be determined by the underlying condition that caused the effusion and fetal status.

If the woman is rhesus negative, anti-D immunoglobulin should be given within 72 hours after the procedure. If the amniotic fluid amount is too low to allow an adequate visualisation for the technique, an amnioinfusion is performed by administering saline through the puncture needle once the amniotic cavity has been reached.

Complications

To date, pericardiocentesis is a safe and effective procedure. The main complications are related to the amnion puncture, like with amniocentesis: temporary loss of amniotic fluid and premature rupture of membranes (PROM). Other rare complications are fetal injuries, vertical transmission of infectious diseases, intrauterine infection, pregnancy loss, and, rarely, septic shock.

Table 2: Reported cases of fetal pericardiocentesis.

Reference	Cause	GE PC (weeks)	Prenatal evolution	Outcome/postnatal evolution
Cavalle-Garrido[29]	CD	20	No recurrence of PE	Full-term live birth. Asymptomatic at 12 months old
Johnson et al.[30]	CD	20	No recurrence of PE	Full-term live birth. Asymptomatic at 16 months old; no treatment
Bernasconi et al.[25]	CD	25	Intrauterine fetal death	Cardiac diverticulum rupture
McAuliffe et al.[31]	CD	16	No recurrence of PE	Full-term live birth. Asymptomatic at 10 months old. No treatment
McAuliffe et al.[31]	CD	14	No recurrence of PE	Full-term live birth. Asymptomatic at 8 months old; no treatment
Prefumo et al.[32]	CD	16	No recurrence of PE	Full-term live birth. Asymptomatic at 22 months old; no treatment
Gardiner et al.[33]	CD	14	No recurrence of PE	Full-term live birth; asymptomatic; no treatment
Carrard et al.[34]	CD	17	No recurrence of PE	Full-term live birth; asymptomatic at 11 months old; no treatment
Abi-Nader et al.[35]	CD	24	No recurrence of PE	Full-term live birth; asymptomatic at 12 months old; no treatment
Barberato et al.[26]	CD	20	Enlargement of PE	Prenatal fetal death at 37 weeks
Williams et al.[36]	CD	18	Relapse 1 week later and subsequent spontaneous resolution at Week 32–33	-
Garcia Rodriguez et al.[13]	CD	17	No PE recurrence	Full-term live birth. Asymptomatic at birth. Healthy at 5 years old
Zeng et al.[37]	CD	20	-	Live birth. Asymptomatic
Zeng et al.[37]	CD	24	-	Live birth. Asymptomatic
Antiñolo et al.[27]	CDH	21, 22+4	No PE recurrence	Caesarean at 37 weeks gestation. EXIT procedure. Thoracic surgery with correction of the defect 2 hours after birth. Asymptomatic at 18 days old
Kanamori et al.[28]	CDH	27	Small PE	Live birth at 36 weeks. Thorax surgery to correct the defect. Asymptomatic at 11 months old
Benatar et al.[9]	T	33	Small PE recurrence	Live birth at 37 weeks. Spontaneous onset of labour. Caesarean for transverse presentation. Cardiac surgery after stabilisation. Healthy at 2 months old
Iacona et al.[18]	T	32, 33, 34	Recurrence of the PE	Live birth at 34 weeks. Induction of labour. Vaginal delivery. No cardiorespiratory compromise after birth
Sklansky et al.[23]	T	24, 27	Recurrence of the PE	Live birth at 35 weeks. Caesarean. Cardiac surgery at birth. Asymptomatic at 12 months old
Bruch et al.[15]	T	28	Recurrence of PE, develop of hydrops,	Live birth at 34 weeks. Caesarean. Cardiac surgery at birth
Paw and Jamieson[20]	T	Multiple PC	Deterioration of the hydropic condition	Live birth at 34 weeks. Caesarean. Cardiac surgery after stabilisation. Asymptomatic at discharge
Fujimori et al.[38]	T	34	Improvement at 3 days after PC. Deterioration of the hydropic condition	Live birth at 37 weeks. Caesarean. Cardiac surgery. Asymptomatic at 6 months old
Sepulveda et al.[17]	T	37, 37+4	Improvement at 4 days after PC but recurrence afterwards	Live birth at 37+4 weeks. Caesarean. Cardiac surgery on the first day. Asymptomatic at 6 days old
Pratt et al.[39]	T	28	-	Live birth at 34 weeks. Caesarean. Cardiac surgery on the first day. Asymptomatic at 3 years old
Laquay et al.[40]	T	34	Deterioration of the hydrops at 2 days	Live birth at 34+2 weeks. Cardiac surgery on the 3 days. Asymptomatic at 3 months old

Table 2 continued.

Reference	Cause	GE PC (weeks)	Prenatal evolution	Outcome/postnatal evolution
Laquay et al.[40]	T	28	-	Live birth at 32 weeks. Spontaneous onset of labour. Caesarean. Cardiac surgery at 3 days old. Asymptomatic at 3 months old
Tollens et al.[41]	T	33	Recurrence of PE and risk of hydrops development	Live birth at 36 weeks. Caesarean. Cardiac surgery after stabilisation. Asymptomatic at 3 years old
Kamil et al.[21]	T	28+2,30+,1+31+1, 32, 32+6	Improvement after PC but recurrence afterwards	Live birth at 34 weeks. Caesarean. PC after delivery. Cardiac surgery 4 days after delivery. Asymptomatic at 12 months old
Czernik et al.[22]	T	27, 28, 29, 31	Improvement at 3 days after PC but recurrence afterwards	Live birth at 31+5 weeks, premature rupture membranes and pathological fetal heart rate tracing. PC after birth. Cardiac surgery at 3 days old
Goldberg et al.[42]	T	31	-	Live birth at 31 weeks. Cardiac surgery on Day 2 of life. Asymptomatic at discharge
Michailidis et al.[43]	CGD	33	Recurrence at 35 weeks	Live birth at 35 weeks. PC after birth. Widespread pustular lesions. Antibiotic treatment
Walsh et al.[44]	KH	34+5	-	Live birth at 38 weeks. Induction of labour. PC after birth. Surgery at 7 weeks old

GE PC: gestational age of the pericardiocentesis; PC: pericardiocentesis; CD: cardiac diverticulum; CDH: congenital diaphragmatic hernia (Morgagni); T: cardiac teratoma; PE: pericardial effusion; CGD: chronic granulomatous disease; KH: kaposiform haemangioendothelioma.

REVIEW OF THE LITERATURE

We performed a PubMed search and reviewed the literature for cases with pericardiocentesis performed prenatally. Patients who underwent drainage by placing shunts additional to pericardiocentesis were excluded, and cases where only pericardiocentesis was performed as a pericardial effusion treatment were studied. Table 2 summarises the cases where pericardiocentesis was performed.

At the time of writing, 33 cases of prenatal intrauterine pericardiocentesis have been reported. In 14 cases, pericardial effusion was associated with cardiac diverticulum, 14 cases to cardiac teratoma, 2 cases to congenital diaphragmatic hernia (intrapericardial hernia or Morgagni's hernia), 1 case of kaposiform haemangioendothelioma, and 1 case of X-linked chronic granulomatous disease. In cardiac diverticula, the pericardial effusion was resolved with a simple pericardiocentesis. For cardiac teratomas eight cases used a single pericardiocentesis to drain the effusion and for six fetuses the use of serial pericardiocentesis was necessary.

There were no complications reported in relation to the pericardiocentesis, but in one case of cardiac teratoma, PROM occurred at 31 weeks. In this fetus a total of four pericardiocenteses were performed: three prior to PROM to improve fetal status associated with hydrops, extending *in utero* life for 4 weeks and allowing fetal lung maturation with corticosteroids at 29 weeks; and one pericardiocentesis performed prior to delivery at 31+5 weeks.[22] Two fetal deaths were reported in association with cardiac diverticulum. In one case this was due to diverticulum rupture[27] and in the other case it occurred 17 weeks after the procedure.[33]

Neonatal survival was high. All live neonates (n=31) were asymptomatic at hospital discharge. In the two cases of intrapericardial congenital diaphragmatic hernia (Morgagni) with pericardial effusion, the procedure avoided the development of pulmonary hypoplasia and avoided neonatal respiratory morbidity and fetal death.[36,37] In conclusion, fetal pericardiocentesis is a safe procedure allowing the possibility of fetal lung maturation and improving fetal extraction with a better haemodynamic and respiratory condition at delivery.

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INHERITED BONE FAILURE SYNDROMES, FOCUS ON THE HAEMATOLOGICAL MANIFESTATIONS: A REVIEW

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ABSTRACT

The purpose of this review is to provide the haematologist with a working knowledge of the common inherited bone marrow failure syndromes (iBMFS) diagnosed in early childhood to young adulthood. Although these disorders are heterogeneous, this article discusses their common features, pathophysiology, and management. Each of these syndromes has a spectrum of clinical variation and can cause both haematological and non-haematological manifestations. Most pathogenic mutations responsible are in genes important to a progenitor cell's ability to maintain genomic integrity, which accounts for the clinical phenotypes often affecting multiple tissues. Furthermore, all of these syndromes predispose not only to aplastic anaemia but also to myelodysplastic syndrome/acute myeloid leukaemia. Since iBMFS only account for a small percentage of childhood leukaemia cases, it is important that the clinician maintains a high clinical suspicion as appropriate diagnosis impacts treatment, health screening, and family members. Identification of iBMFS is critically important for appropriate donor selection and transplant regimens, as haematopoietic stem cell transplantation is curative for the haematological manifestations of these diseases, but treatment-related mortality can be excessive if modifications are not made to conditioning.

Keywords: Inherited bone marrow failure syndromes (iBMFS), Fanconi anaemia (FA), dyskeratosis congenita (DS), Shwachman–Diamond syndrome (SDS), Diamond–Blackfan anaemia (DBA), leukaemia, myelodysplastic syndrome (MDS), inherited susceptibility, germline mutations.

INTRODUCTION

Inherited bone marrow failure syndromes (iBMFS) are a heterogeneous group, with variable phenotypes, inheritance, and gene mutations, but also with striking similarities. All iBMFS include failure of adequate blood cell production, and whilst some are lineage specific, most lead to progressive pancytopenia. Many of the iBMFS are associated with a decreased number of CD34+ haematopoietic progenitor cells (HPC), along with stress erythropoiesis, and a predisposition to myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML). iBMFS are often characterised by multiple non-haematological findings, such as congenital malformations and predisposition to solid tumours. Variability exists and even the same mutation in individuals within one family can vary from severe phenotypic findings to being clinically silent. Although the mutated genes vary between syndromes, most are key to cellular homeostasis and vital to dividing progenitor cells.[1]

When Dr Fanconi published a landmark paper in 1927 describing families with severe bone marrow failure (BMF) in conjunction with multiple congenital anomalies, it was the clinical phenotype that elucidated the diagnosis of Fanconi anaemia (FA).[2] In the modern era, with improved diagnostic tools, such as clastogenic assays and gene panel sequencing, it is now apparent that FA and other iBMFS also often occur in patients with less severe clinical presentations. Accordingly, the prevalence of iBMFS is likely to be higher than the estimate

of 65 per million.[3,4] Today, we recognise that diagnosis of iBMFS is not just made in young children with multiple congenital anomalies and that for patients with subtler physical findings the diagnosis can be delayed to adolescence/young adulthood (AYA) during the evaluation of newly diagnosed aplastic anaemia (AA) or MDS/AML. In a study of patients who had a haematopoietic stem cell transplant (HSCT) for AA or MDS, germline mutations diagnostic of a iBMFS were identified in 5% and 14% of patients, respectively, and neither family/personal history or physical stigmata were predictive.[5] Physical anomalies were present in less than a third of the patients. The peril of under-diagnosis is not only that individuals have specific health risks, but if they remain undiagnosed at the time of MDS/AML presentation they are at risk from severe toxicity, if 'standard' chemotherapy regimens are not adjusted appropriately.[6-8] Moreover, although we now routinely test for many of the somatic mutations and cytogenetic abnormalities to inform risk-adjusted treatments during the evaluation of AML/MDS, a comprehensive evaluation of genes associated with iBMFS is not routine. Thus, recognising when to evaluate further for possible hereditary predispositions remains of great importance.[3,9]

FANCONI ANAEMIA

In addition to a young child with pancytopenia, well-described characteristics suggesting FA include hypoplastic thumb, radial ray skeletal defects, café-au-lait spots, microcephaly, and undescended testicles. Other non-haematological manifestations include short stature, characteristic facial findings, skeletal, renal, gut, gonadal, urogenital, cardiopulmonary, and central nervous system anomalies, as well as neurocognitive delay. Such findings direct the haematologist to order a chromosomal fragility test in the presence of DNA crosslinking agents, diepoxybutane or mitomycin C; the gold standard for FA diagnosis. Occasional false positives may occur in patients with other chromosomal breakage syndromes, such as Nijmegen breakage syndrome. False negative results on blood analysis may occur due to genetic reversion of the HPC, wherein spontaneous resolution of pancytopenia can occur through the repair of a mutated allele.[10] In the setting of a high suspicion for FA, a germline mutation assay on skin fibroblasts should be performed. Identification of pathogenic FA mutations on genetic analysis is confirmatory. Clinical suspicion is important due to variable penetrance and expressivity during evaluation of an AYA with AA or MDS/AML. Approximately 10% of FA patients will not be diagnosed until they are 16 years of age or older.[11] Congenital anomalies are absent in ~40% of patients. Therefore, part of the diagnostic workup for AML or MDS should include a detailed family/personal history and careful physical exam to look for specific findings. It is easy to overlook manifestations, such as short stature, since a patient may fall within the normal range unless one calculated the patients' predicted height based on mid-parental height. Diagnosis of FA is critically important in patients with AA or MDS/AML, because the hallmark of the syndrome is genomic instability in the presence of DNA crosslinking agents and treatment plans for these diseases typically include such agents as part of a HSCT. Furthermore, dose attenuation is required to decrease treatment-related mortality (TRM) and morbidity.[12] Lastly, diagnosis allows for screening of other FA-associated complications, and is important for family planning.

FA is primarily of autosomal recessive inheritance, although there are autosomal dominant (all *de novo*) and X-linked inheritance as well. Currently, 21 genes have been identified that, when mutated, cause FA,[13] with biallelic *FANCA* mutations the most common genetic aetiology. These genes are critical for the cells to repair damage caused by DNA interstrand crosslinks normally by homologous recombination. Mutations in the FA complex increase the alternate repair pathway (non-homologous recombination), which is more error prone, leading to genomic instability.[1,13,14] Since in FA DNA damage is mediated by exposure, the fact that the HPC is exposed to endogenous toxins, such as oxidative stress and aldehydes, also makes it particularly sensitive.[15] The DNA damage in these dividing HPC activate p53 and like pathways that lead to cell cycle arrest and either apoptosis or senescence to prevent propagation of mutations in the daughter cells, but this causes an accelerated attrition rate of HPC. In fact, FA patients have low numbers of CD34+ HPC.[16] This is similar to ageing, but here the blood ages at an accelerated rate. A vicious cycle occurs of the marrow in FA patients because the HPC death triggers inflammation and mitochondrial damage, increasing reactive oxygen species further accelerating this process.[17] This explains the progressive BMF.[18] Functional reversion, where the pathogenic mutation is 'reversed' by a new somatic mutation leading to normal phenotypic function,

occurs in ~10–25% of patients. This gives rise to a reverted HPC, which has competitive advantage over the non-reverted FA HPC, allowing for spontaneous remission of the cytopenias.[19] Mosaicism of the HPC in blood and marrow may result in false negatives on blood chromosomal fragility testing; therefore, in patients where there is high clinical suspicion, testing should be performed on skin fibroblasts. These patients remain at risk of MDS/AML by non-reverted HPC, and, since non-haematological tissue is not repaired, their risk of solid tumours remains unchanged.

The cumulative incidence of MDS and AML before the age of 40 years is 30% and 10%, respectively. The risk of cancer in FA is >500-times that in the normal population, with AML being the most frequent malignancy, although a wide range of solid tumours may also be diagnosed.[20] Although FA patients are young when they present with AML, they do not typically have the favourable, reciprocal translocations associated with young patients with *de novo* AML, but instead they have multiple chromosome deletions and additions more typical of MDS/AML seen in older individuals or those with therapy-related disease. These include unbalanced gains in the long arms of chromosomes 1 and 3 and deletions in chromosomes 7, 11, and 21.[21] Additionally, *RUNX1* somatic mutations are seen in 20% of patients with FA and AML and are also associated with poor survival.[22] When evaluating MDS/AML in younger patients, a history of progressive cytopenias and/or high-risk or complex cytogenetics should make the clinician consider an underlying diagnosis of FA.

The only current curative treatment for the haematological manifestations of FA is HSCT. The first attempt at HSCT for FA resulted in extreme TRM, but by the early 1990s studies showed survival could be improved by lowering the dosage of radiation and alkylating agents. A decade later, in the setting of lower dose total body irradiation (TBI) and cyclophosphamide, matched sibling donor (MSD) transplants, overall survival (OS) of >85% were reached, but with dramatically increased risks of non-haematological cancers in survivors.[12,23,24] Current approaches use fludarabine-based reduced intensity conditioning (RIC), which allow for engraftment without high dose alkylating agents and TBI. This has further attenuated the TRM, and FA patients without MDS/AML now have ~90% 5-year survival with a MSD transplant.[25,26] A MSD who does not share the pathogenic mutation of both alleles is preferred. All full siblings should be human leukocyte antigen-typed and undergo FA testing regardless of phenotypic evidence of disease. A recent study was undertaken of FA patients without AML who were transplanted with an unrelated donor or haploidentical donor, without radiation and using a T cell deplete graft. This study had a 3-year OS of 80% without significant graft versus host disease (GVHD). Non-relapse mortality was 18%, infection being the most common cause.[27] FA, HSCT, and GVHD are all independent risk factors for mucocutaneous human papilloma virus (HPV)-related squamous cell carcinomas (SCC); thus, FA patients who have been transplanted remain at high risk and require ongoing surveillance.[28,29] The rationale of this study was to mitigate this risk of SCC by giving radiation-free conditioning and lowering the incidence of GVHD by T cell depletion. The last decade has seen major advancements in our understanding of the pathogenesis and the implications of diagnosis on treatment, resulting in improved patient outcome. Newer approaches include gene therapy trials, where the pathogenic mutation is corrected by *in vitro* transduction of the patient's HPC. Treatment of overt leukaemia remains challenging, because treatment intensity and pre-transplant cytoreduction needed to cure AML can result in unacceptable non-relapse mortality; current strategies include sequential timing and/or addition of cytarabine.[21,30] The treatment of AML is further evolving to include immunotherapy and targeted agents with less cytotoxicity and has the potential to further improve patient outcomes within the next decade.

DYSKERATOSIS CONGENITA

Dyskeratosis congenita (DC) is a heterogeneous disorder with variable modes of inheritance: X-linked, autosomal dominant, and autosomal recessive.[1,31] Families may demonstrate genetic anticipation, where early generations have less severe disease and clinical manifestations are more apparent at earlier ages in subsequent generations.[32] Patients with classic DC present with the triad of skin/nail dystrophy, reticular pigmentation of the skin, and mucosal leukoplasia in early childhood.[33] Other manifestations include microcephaly, developmental delay, teeth abnormalities, lacrimal duct stenosis, exercise intolerance, oesophageal stenosis, SCC of the head, neck, and/or anogenital region, liver disease, hair loss, premature greying, osteoporosis, combined immunodeficiency, ataxia, and pulmonary

arteriovenous malformations.[1,33,34] More than 90% of individuals with DC will develop cytopenias by the age of 40 years. The classic presentation defines only a fraction of patients who primarily have mutations in the X-linked gene *DKC1*. Others with DC have BMF without mucocutaneous findings, isolated pulmonary and/or hepatic fibrosis, or are phenotypically silent. As with other iBMFS, this variation makes it a diagnostic challenge. Because the mucocutaneous and nail findings can mimic chronic GVHD, an atypical presentation of DC may be considered in patients transplanted for AA with what is thought to be severe GVHD.[35] Pathologic mutations in 13 genes have been identified, all of which interfere with telomere maintenance; however, approximately one-third of DC remains genetically unclassified.[31] Mutations in *TINF2*, *RTEL1* (autosomal recessive form), and in the catalytic domain of DKC1 are associated with the more severe phenotypes; whereas mutations in *TERC* and *TERT* are often diagnosed later with BMF/AA and/or fibrosis. The diagnosis may be established by clinical findings and confirmed by telomere length measurements in leukocyte subsets, such as by flow cytometry fluorescence *in situ* hybridisation (flow-FISH).[31,36] Flow-FISH is 95% specific and nearly 100% sensitive in patients with a telomere length <1% of age-matched controls.[37]

DC also demonstrates a premature ageing phenotype with early attrition of progenitors. Maintaining adequate telomere length is important in dividing progenitors in all tissues. Therefore, it is not surprising that most of the tissues severely affected have more actively replenishing progenitors, such as blood, skin, and mucus membranes. Pathogenic mutations in DC decrease the function of telomerase complex, disabling the cell from being able to maintain its telomere length and results in prematurely shortened telomeres.[38] Telomeres are structures made from tandem DNA repeats and associated proteins that cap the ends of chromosomes and protect them from degradation.

Table 1: When to consider evaluation for inherited bone marrow failure syndromes in a young patient with cytopenias, myelodysplastic syndrome, or acute myeloid leukaemia.

	FA	DC	DBA	SDS	*RUNX1*	*GATA2*
Personal/family history						
Family history of AA, MDS, AML	✓	✓	✓	✓	✓	✓
Parental consanguinity	✓	✓		✓		
Progressive cytopenia/thrombocytopenia	✓	✓	✓	✓	✓	
Head/neck/anogenital squamous cell carcinoma	✓	✓				
Increased toxicity with cytotoxic therapy	✓	✓		✓		
Pulmonary fibrosis		✓				
Cirrhosis of the liver of unknown aetiology		✓				
Cytopenia that had spontaneous remission	✓	✓	✓			
Clinical features						
Congenital anomalies	✓	✓	✓	✓		✓
Dysmorphic features, short stature (height <5 cm below sex specific mid-parental estimate)	✓	✓	✓			
Leukoplakia, oesophageal stricture		✓				
Hyper/hypopigmentation, café-au-lait macules	✓	✓				
Alopecia, premature hair greying	✓	✓				
Neurocognitive delay	✓	✓	✓	✓		
Immunodeficiency		✓				✓
Laboratory findings						
Macrocytosis/increase HbF	✓	✓	✓	✓		
Hypocellular marrow (without diagnosis of AA)	✓	✓				
MDS/AML with -7/-7q and/or other complex cytogenetics	✓	✓			✓	✓

AA: aplastic anaemia; AML: acute myeloid leukaemia; DC: dyskeratosis congenital; DBA: Diamond–Blackfan anaemia; FA: Fanconi anaemia; HbF: haemoglobin F; MDS: myelodysplastic syndrome; SDS: Shwachman–Diamond syndrome.

Telomere ends that have shortened below a critical length signal that a cell has reached the end of its replicative potential and the cell becomes senescent or apoptotic. In DC, progenitors lose full replicative potential, undergoing early attrition.

Patients with DC have malignant predisposition, particularly for SCC and MDS/AML. Compared to the general population, patients with DC have a 195-fold increased risk of AML, a 2,362-fold increased risk of MDS, particularly hypocellular MDS, and a 1,154-fold increase in the risk of head and neck squamous cell cancers.[39] By the age of 50 years old, the risk of malignancy is ~20–30%, for the majority solid cancers. As previously stated, cells with shortened telomeres activate p53 and undergo replicative senescence, but if the cell has developed a premalignant somatic mutation (such as in p53) then the cell will be unable to respond to these signals to arrest, and will divide until very short telomeres cause a telomere crisis. This has two different outcomes: an appropriate response of cell death, or the cell acquires a second hit, another somatic mutation which may cause immortalisation and cancer. Furthermore, shortened telomeres increase genomic instability due to end-to-end fusions and that genes involved in telomere maintenance are also involved in DNA repair.[40] While DC is more rarely associated with MDS or AML in children compared to FA, it must be considered along with other iBMFS during the evaluation of young people with MDS/AML, particularly as serious treatment-related toxicity is often seen with standard cytotoxic leukaemia regimens.

The only curative treatment for haematological manifestations is HSCT. MSD transplant with conventional radiation-based conditioning is associated with high TRM.[41] The clinical sequelae of some chemotherapy agents, radiation, and GVHD overlaps with the natural clinical progression of DC and are independent risk factors for SCC, restrictive lung disease, and liver disease. Treatment therefore amplifies the risk of secondary cancers. Patients with DC and pre-existing pulmonary fibrosis and/or hepatic cirrhosis are more susceptible to TRM. RIC-HSCT is preferred and the agents that cause lung and liver toxicity, such as busulfan and high dose TBI, should be avoided.[42-45] The 5-year OS with this approach is ~70%, with patients >20 years old having significantly worse outcomes than younger patients.[43] If radiation is required, then lungs should be blocked from the field. The preferred donor in DC is a MSD and, because clinical findings may be absent, it is important to screen all potential family donors for the gene mutation and/or by telomere assay. The chance that a sibling is both fully matched and unaffected is ~12%.

RIBOSOMOPATHIES: SHWACHMAN–DIAMOND SYNDROME AND DIAMOND–BLACKFAN ANAEMIA

Ribosomes are the complexes responsible for the translation of mRNA and are a critical component of cellular machinery. Gene mutations that result in ribosome dysfunction occur in several iBMFS.[46] Like other iBMFS, there is clinical heterogeneity, but common findings include cytopenias, congenital anomalies, stress erythropoiesis, and a predisposition to cancer including MDS/AML. These syndromes are more easily diagnosed in a patient with a classic constellation of symptoms in childhood, but, because not all individuals will have the classic manifestations, some may escape diagnosis until later when they present with MDS.

Two of the classic syndromes are Shwachman–Diamond syndrome (SDS) and Diamond–Blackfan anaemia (DBA). SDS is a recessive disease due to biallelic mutations of *SBDS*. Affected children classically present in early childhood with exocrine pancreatic insufficiency, neutropenia, congenital anomalies, dystrophy, and skeletal abnormalities.[47] Although neutropenia is the most common manifestation of SDS, all lineages can be affected. DBA is caused by heterozygous mutations of 12 ribosomal genes with variable inheritance patterns, including autosomal dominant with haploinsufficiency of *RPS19* (most common), X-linked, and autosomal recessive. Patients with DBA present with pure red cell aplasia and congenital craniofacial, skeletal, renal, and/or cardiac anomalies, in the first year of life.[48] Anaemia, reticulocytopenia, macrocytosis, and a marrow deplete of erythroid progenitors are diagnostic of DBA.[1,48] Although there is no clear genotype-phenotype correlation for the severity of the cytopenia, the non-haematological manifestations tend to segregate with the pathogenic mutations. Thumb abnormalities are virtually absent in patients with *RPS19* mutations but are frequently present in those with *RPL5* or *RPL11* mutations.[1,49] The X-linked form is rare and only two families have been identified, but is unique in that the gene mutated is not a ribosomal gene but *GATA1*, encoding a transcription factor. These patients only express haematological manifestations, although

not elevated ADA. Elevated ADA distinguishes patients with DBA from transient erythroblastopenia of childhood, so knowledge of this exception is important as the patients with *GATA1* mutations are at risk of MDS/AML.[50] Increasingly, these syndromes are being recognised in AYA who have had mild manifestations or spontaneous remission of the anaemia earlier in life and not recognised until they present with MDS/AML or another cancer. The cumulative incidence of MDS/AML in SDS is estimated to be ˜10% by the age of 20 years, most frequently involving deletions of chromosome 7. This is in contrast to the estimated occurrence of MDS/AML, thought to be <2% of patients with DBA, although this may be higher in those with *GATA1* mutations.[51,52] DBA patients also have an increased risk of other cancers with an overall risk of malignancy estimated to be 20%.[52] HSCT is curative for patients with SDS and DBA who have severe haematological manifestations. Patients with SDS experience increased toxicity with transplant, and RIC-HSCT should be considered.[53] Patients with DBA can have excellent outcomes with MSD-HSCT, and results with unrelated donor-HSCT are improving. Iron overload is a risk in patients that are heavily transfused from an early age and should be evaluated and treated prior to transplant. All potential related donors should be genetically screened.

GERMLINE MUTATION OF MASTER PROGENITOR REGULATORS: *GATA1/GATA2/RUNX1*

Like *GATA1*, *GATA2* also encodes a transcription factor and germline mutations have been associated with cytopenias and predisposition to MDS/AML.[54] *GATA1* germline mutations cause a spectrum of cytopenias from isolated anaemia (DBA), to thrombocytopenia, or rarely pancytopenia. Germline mutations of *GATA2* have historically been described as two classic immunologic syndromes. Emberger syndrome is described as lymphoedema, sensorineural hearing loss, warts (HPV-associated), low CD4/CD8 ratio, and MDS; monocytopenia and mycobacterial infection syndrome is described as monocytopenia and predisposition to viral/nontuberculous mycobacterial infections. Autosomal dominant inheritance or *de novo* mutations of germline *GATA2* have a variable phenotype and other features may include neutropenia, lymphopenia (B lymphocytes and natural killer cells), BMF, MDS/AML, Epstein–Barr virus-associated cancers, pulmonary alveolar proteinosis, neurocognitive delay, and erythema nodosum.[54-57] An elevated peripheral CD34 count is often present at the onset, but declines with declining blood counts. *GATA2* is now recognised as the most common germline mutation to cause MDS/AML in younger patients. It has recently been reported to account for 9% of all primary MDS cases in children <18 years of age, and 37% of young patients (72% of patients ages 12–19) with monosomy 7 MDS.[5] Therefore it is recommended that all young patients with primary MDS with monosomy 7 should undergo germline *GATA2* testing. HSCT is the only curative option. Not only is AA, MDS, and/or AML an indication for transplant, transplant in patients with germline *GATA2* mutations has also been shown to improve the underlying immunodeficiency and pulmonary alveolar proteinosis.

RUNX1 is a haematopoietic transcription factor that is upregulated after the progenitor cell undergoes megakaryocytic differentiation. Germline mutations of *RUNX1* cause autosomal dominant familial platelet disorder with associated myeloid malignancies (FPD/AML).[58] These patients often experience lifetime bleeding tendencies secondary to moderate thrombocytopenia and functional platelet defects. An estimated 35–40% of these patients will develop AML typically as an AYA; the median age of diagnosis is 33 years of age, although patients who develop AML in the first year of life through to their mid-60s have also been described. T cell acute lymphoblastic leukaemia has also been described in these families. Patients with dominant negative mutations have a higher propensity to develop leukaemia. Not all patients will present with bleeding and may go undiagnosed until the development of MDS/AML. This syndrome also appears to be associated with genetic anticipation.[58-61]

CONCLUSION

Phenotypic and genotypic variation of the iBMFS make diagnosis challenging. It is important for the clinician to maintain a high level of suspicion when evaluating younger patients with AA, MDS/AML, since treating with chemotherapy or HSCT without knowledge of an underlying diagnosis leads to poor outcomes. Table 1 outlines some of the findings that can suggest diagnosis of iBMFS. Taking a detailed family and personal history, careful physical exam, and thorough review of laboratory and cytogenetic findings can assist the clinician in determining when

to pursue testing and genetic counselling referral.[3] This will lead to better patient outcomes and allows adherence to syndrome specific guidelines.[6-8] HSCT remains the mainstay of treatment for these patients and diagnosis is an important factor in choosing pre-transplant therapy, conditioning regimen (agents and intensity), donor type, GVHD prevention strategy, and survivorship screening.

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NON-CLASSIC MYELOPROLIFERATIVE NEOPLASMS: ARE WE REALLY AWARE OF THESE RARE DISEASES IN DAILY PRACTICE?

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ABSTRACT

Chronic neutrophilic leukaemia (CNL), chronic eosinophilic leukaemia-not otherwise specified (CEL-NOS), and myeloproliferative neoplasm (MPN), unclassifiable are rare clonal diseases, known as 'non-classic myeloproliferative neoplasms'. They are diagnosed largely based on exclusion of underlying reactive causes by patient history, physical examination, serological tests, and imaging studies. As well as peripheral blood testing, bone marrow examination is mandatory to exclude bone marrow infiltrating conditions such as multiple myeloma, acute leukaemias, etc. Today, molecular genetic classification should be undertaken to establish accurate diagnosis, in addition to the traditional morphological classification of MPN. Therefore, molecular genetic testing should take part in the diagnostic work-up of suspected patients with rare MPN. Of CNL patients, 90% (and in some datasets 100%) have mutations in *CSF3R*, which has led to the addition of this finding to the diagnostic criteria for CNL. The absence of rearrangements of *FIP1L1/PDGFRA*, *PDGFRA*, *PDGFRB*, *FGFR1*, and *PCM1-JAK2* fusions should prompt consideration of a diagnosis of chronic eosinophilic leukaemia-not otherwise specified. MPN, unclassifiable, the least frequent type, is considered when an MPN has definite MPN features but does not meet diagnostic criteria for either the classic or the other non-classic MPN. They all share common symptoms and findings. Transformation to acute leukaemia is still a major clinical problem. Since no standard of care exists, the treatment approach is still symptomatic for all. This is an indicator that we really need disease-modifying drugs against initial diagnostic molecular markers, such as *CSF3R* inhibitors, which might change the natural history of these disorders. Therefore, participation in clinical trials is mandatory for this extremely rare patient population.

Keywords: Rare myeloproliferative neoplasms, chronic neutrophilic leukaemia (CNL), chronic eosinophilic leukaemia-not otherwise specified (CEL-NOS), myeloproliferative neoplasm, unclassifiable (MPN-U).

INTRODUCTION

Myeloproliferative neoplasms (MPN) are rare clonal haematologic diseases characterised by the proliferation of mature blood elements from several myeloid cell lines. The more frequently encountered chronic MPN include polycythaemia vera, essential thrombocythaemia (ET), chronic myeloid leukaemia (CML), and primary myelofibrosis (PMF), respectively. It is important to emphasise that an accurate diagnosis is mandatory for management of and in predicting prognosis in this group of diseases. Today, the traditional morphological classification of MPN is replaced by molecular genetic classification. The main driver mutations in MPN include Janus Kinase 2 (*JAK2*), calreticulin (*CALR*), and myeloproliferative leukaemia virus oncogene (*MPL*). The presence of a mutation associated with an MPN subtype is specific for diagnosis; however, the absence of *JAK2*, *MPL*, or other related mutations is not sufficient to exclude the diagnosis of MPN. Common findings of MPN include organomegalia, increased metabolism related constitutional symptoms, and hypercellular bone marrow (BM) with blast percentage <20%. The primary treatment goals in MPN are to avoid thrombosis and bleeding, treat MPN-related symptoms, improve quality of life, and minimise the

risk of malignant transformation and myelofibrosis. Leukaemic transformation is associated with adverse clinical outcomes, characterised by a poor response and early resistance to conventional therapies. Current MPN clinical research is focussed on the development of prognostic biomarkers and development of drugs that can modify disease natural history.

In 2016, the World Health Organization (WHO) revised the classification of MPN (Table 1).[1] The most seen classical MPN (polycythaemia vera, ET, CML, and PMF) are beyond the scope of this review, and will not be discussed.[2-4] Namely, the less frequently encountered ones (chronic neutrophilic leukaemia [CNL], chronic eosinophilic leukaemia-not otherwise specified [CEL-NOS], and myeloproliferative neoplasm, unclassifiable [MPN-U]) will be reviewed extensively.

CHRONIC NEUTROPHILIC LEUKAEMIA

CNL is a rare MPN, of which approximately 150 cases have been described to date; however, only 40 'true' cases meet the criteria of the last WHO classification.[5] The median age is 65 years (26–83 years) at diagnosis, with male predominance.[6] The clinical course of CNL is heterogeneous. However, one can state that the disease can be subdivided as chronic phase, accelerated phase, and blast phase, similar to CML.[6] An accelerated phase can be considered if progressive neutrophilia, progressive splenomegaly, or worsening thrombocytopenia develop with resistance to previously effective therapy. A blast phase can be considered if BM blast percentage is >20%. Blastic transformation (mostly reported as myeloid) occurs in a significant proportion (˜20%) at a median of 21 months from diagnosis.[7]

Table 1: World Health Organization (WHO) 2016 classification of myeloproliferative neoplasms.[1]

- Chronic myeloid leukaemia, *BCR/ABL1*-positive
- Chronic neutrophilic leukaemia
- Polycythaemia vera
- Primary myelofibrosis
  - Primary myelofibrosis, prefibrotic/early stage
  - Primary myelofibrosis, overt fibrotic stage
- Essential thrombocythaemia
- Chronic eosinophilic leukaemia-not otherwise specified
- Myeloproliferative neoplasm, unclassifiable

Other definitions that do not fit the accelerated phase and blast phase can be considered as chronic phase.

The majority of patients are asymptomatic at diagnosis, and leukocytosis is detected incidentally in routine laboratory tests. Fatigue is the most common presenting symptom. Other symptoms reported include weight loss, easy bruising, bone pain, and night sweats. The most frequent finding is splenomegaly.[7] Splenomegaly may range from mild to massive, and may be related to weight loss, early satiety, and abdominal fullness. Hepatomegaly and lymphadenopathy are uncommon at presentation.[8] Skin findings are rare, but may be presented as leukaemia cutis, Sweet's syndrome, and pyoderma gangrenosum.

The principal feature of CNL is neutrophilic leukocytosis that is characterised by toxic granulation and Döhle bodies in the absence of prominent immature granulocytosis and disgranulopoiesis. Many patients tolerate very high leukocyte counts without having any symptoms or direct evidence of end organ damage. Varying degrees of anaemia and thrombocytopenia are present. Bleeding diatheses are common in CNL due to acquired von Willebrand's disease and other acquired coagulation factor or platelet function defects. Leukocyte alkaline phosphatase and vitamin B12 levels may be normal or elevated. BM aspirates and biopsies show a myeloid hyperplasia (>90% cellularity) with an increased myeloid:erythroid ratio, which may reach >20:1, and myeloblasts represent <5% of the cells. Erythropoiesis and megakaryopoiesis are typically normal, and dyshaematopoiesis is not present in any cell lineage. Reticulin fibrosis is not significantly increased.

It is diagnosed largely based on exclusion of underlying causes of reactive neutrophilia and/or the lack of specific molecular markers of other haematological malignancies.[9] The diagnostic criteria for CNL are displayed in Table 2. Incorporation of molecular studies is helpful to distinguish between benign versus malignant causes of leukocytosis. Colony stimulating factor 3 receptor (CSF3R) is a trans-membrane protein which plays a prominent role in the growth and differentiation of granulocytes.[9] More recently, it was observed that CSF3R is recurrently mutated in patients with CNL,[10] which has led to the addition of this finding to the diagnostic criteria for CNL in the most recent 2016 WHO classification of MPN.[1]

Table 2: Diagnostic criteria for chronic neutrophilic leukaemia.[1]

1.	Peripheral blood WBC ≥25×10^9/L - Segmented neutrophils plus band forms ≥80% of WBC - Neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes) <10% of WBC - Myeloblasts rarely observed - Monocyte count <1×10^9/L - No dysgranulopoiesis
2.	Hypercellular bone marrow - Neutrophil granulocytes increased in percentage and number - Neutrophil maturation appears normal - Myeloblasts are <5% of nucleated cells
3.	Not meeting WHO criteria for *BCR/ABL1* positive CML, PV, ET, or PMF
4.	No rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1*, or *PCM1-JAK2*
5.	Presence of *CSF3R-T618I* or other activating *CSF3R* mutation OR In the absence of a *CSF3R* mutation, persistent neutrophilia (≥3 months), splenomegaly, and no identifiable cause of reactive neutrophilia including absence of a plasma cell neoplasm or, if present, demonstration of clonality of myeloid cells by cytogenetic or molecular studies.

CML: chronic myeloid leukaemia; ET: essential thrombocythaemia; PMF: primary myelofibrosis; PV: polycythaemia vera; WBC: white blood cells; WHO: World Health Organization.

The most common acquired mutation in *CSF3R* is an activating point mutation in the membrane proximal region (*CSF3R*-T618I) which activates JAK/STAT signalling, so is an oncogenic driver. Ninety percent (in some datasets 100%) of CNL patients have mutations in *CSF3R*.[7] Other much less common activating point mutations include T615A and T640N.[10] *CSF3R* mutations could be determined by molecular techniques, such as next-generation sequencing of DNA from the BM aspirate and peripheral blood. CSF3R is known to signal downstream through both JAK and SRC tyrosine kinase pathways.

Having a documented *CSF3R* mutation may help to establish a diagnosis of CNL, but the absence of a *CSF3R* mutation does not rule out CNL. However, *CSF3R*-T618I is a highly sensitive and specific molecular marker for CNL. Also, mutations in *SETBP1* have been demonstrated in 25% of CNL cases.[11] Elliott et al.[12] showed that 57% of patients experienced *ASXL1* mutations. In another study, Elliott and Tefferi[5] concluded that the presence of *ASXL1* mutations and thrombocytopenia at diagnosis were independently predictive of shortened survival. Additionally, CNL may occur with monoclonal gammopathy of undetermined significance in ˜33% of cases.[6] In cases where the BM shows a plasma cell dyscrasia, it is important to prove the neutrophilic clonality by cytogenetic and/or molecular tests.

CNL should be distinguished from atypical CML (aCML). The diagnosis of aCML is strictly based on morphologic evaluation of the blood and marrow, and there are no specific recurring cytogenetic findings that define aCML. However, the frequency of *CSF3R*-T618I mutations in aCML ranges from 0–40%.[11] Neutrophil precursors are >10% of white blood cells, and monocytosis may be seen in aCML. Another important defining feature in aCML is prominent granulocytic dysplasia. So, a BM evaluation and cytogenetic analysis in suspected cases are required to distinguish these disorders.

CNL is associated with a poor prognosis without established standards of care. Therapeutic options include hydroxyurea, JAK1/2 inhibitor (ruxolitinib), imatinib, or dasatinib, interferon-alpha (IFN-α), hypomethylating agents, thalidomide/lenalidomide, histone deacetylase inhibitors, and chemotherapeutic agents (cytarabine and daunorubicin) with very limited data on therapeutic responses. These agents may improve blood counts, but exhibit no proven disease-modifying benefit. Hydroxyurea is the most commonly used drug and is effective in controlling leukocytosis and splenomegaly.[7] Any response to subsequent therapy for hydroxyurea resistance or refractory disease with various agents is often short-lived.[7] No haematologic complete remission has been reported to date following standard induction therapy for accelerated or blast phase in CNL.

Splenectomy is not recommended for management of disease, because it has a high perioperative mortality rate and has been associated with accelerated neutrophilia. Myeloablative or reduced intensity conditioning regimens followed by allogeneic stem cell transplantation (ASCT) is the only curative option for a minority of patients, because of the rarity of this disease, and the older age of many affected. It has been shown that *CSF3R* truncation mutations (Y752X, D771fs, S783fs) operate predominantly through SRC kinases, and exhibit drug sensitivity to SRC kinase inhibitors, such as dasatinib, whereas, *CSF3R* membrane proximal mutations (T618I, T615A) strongly activate the JAK/STAT pathways and are sensitive to JAK kinase inhibitors, such as ruxolitinib.[13-15] I suggest using hydroxyurea to control leukocytosis and for evaluation of eligible patients for ASCT. ASCT may result in favourable long-term outcomes in selected patients, particularly when undertaken in the chronic phase of disease, because those who received it in the accelerated phase died after the procedure.[16-18] Common causes of death include intracerebral haemorrhage, progressive BM failure and related complications, blastic transformation, and progressive multi-organ failure. Median time to acute myeloid leukaemia (AML) transformation is 21 months (3–94 months) and median survival is 23.5 months (1–106 months).[7]

CHRONIC EOSINOPHILIC LEUKAEMIA-NOT OTHERWISE SPECIFIED

CEL-NOS is an extremely rare disorder which is due to clonal proliferation of eosinophil precursors. Its true incidence is unknown. The upper limit of normal for absolute eosinophil count (AEC) is ~600/mm^3.[5] The severity of eosinophilia can be divided into mild (AEC from the upper limit of normal to 1.500/mm^3), moderate (AEC 1.500–5.000/mm^3), and severe (AEC >5.000/mm^3).[19]

The most common presenting symptoms are weakness, fatigue, weight loss, night sweats, persistent cough, dyspnea, pruritus, myalgias, rash, fever, rhinitis, and diarrhoea. Patients with CEL-NOS are predominantly male, and splenomegaly is the most frequent clinical manifestation. Other haematologic findings include neutrophilia, basophilia, myeloid immaturity, and both mature and immature eosinophils with varying degrees of dysplasia in peripheral blood or BM.

In general, all organ systems may be susceptible to the effects of sustained eosinophilia. Progressive heart failure is the most important eosinophil-mediated organ injury. Eosinophil infiltration of cardiac tissue and release of toxic mediators from eosinophils lead to endocardial damage with resulting mural thrombi and increased embolic risk. In the later fibrotic stage, fibrous thickening of the endocardial lining causes a restrictive cardiomyopathy.[20] Valvular insufficiency results from mural endocardial thrombosis and fibrosis involving leaflets of the mitral or tricuspid valves.[21] Besides the heart, lungs, gastrointestinal system, central nervous system, and skin, any other organ may be affected by eosinophil-mediated cytokines.

The diagnostic work-up should begin with eliminating the secondary aetiologies. Secondary eosinophilia has numerous causes. Infections, particularly tissue-invasive parasites, are the most common underlying cause of eosinophilia.[21] The patient's travel history, repeated parasite testing, stool culture, and antibody testing for specific parasites are mandatory for identifying infectious aetiologies. Other potential causes include allergic and hypersensitivity conditions, drug reactions, collagen-vascular diseases (e.g. Churg-Strauss syndrome, systemic lupus erythematosus), pulmonary eosinophilic diseases (e.g. allergic bronchopulmonary aspergillosis), and metabolic conditions (e.g. renal deficiency).[22-24] Also, solid tumours such as T-cell lymphomas, Hodgkin's disease, and acute lymphoblastic leukaemias may be associated with secondary eosinophilia by the production of cytokines such as interleukin (IL)-3, IL-5, and GM-CSF, which promote eosinophil differentiation and survival.[25-27] Therefore, chest X-ray, electrocardiogram and echocardiography, computed tomography scan of the chest/abdomen/pelvis, pulmonary function tests, bronchoscopy, and serologic tests (e.g. aspergillus immunoglobulin E) may be additional laboratory investigations. The diagnostic criteria for CEL-NOS are displayed in Table 3.[28]

If secondary causes of eosinophilia are excluded, one should proceed to the evaluation of a primary BM disorder. Examination of the blood smear and blood tests (e.g. circulating blasts, dysplastic cells, monocytosis, serum B12, or tryptase level) in conjunction with BM analysis will help to discriminate systemic mastocytosis, CML, AML, myelodysplastic syndrome (MDS), aCML, or chronic myelomonocytic leukaemia. In this context, laboratory evaluation of primary eosinophilia should begin with screening of the peripheral blood for the

FIP1L1-PDGFRA gene fusion by reverse transcription polymerase chain reaction (RT-PCR) or fluorescent *in situ* hybridisation (FISH). *FIP1L1/PDGFRA* rearrangement is the most common molecular abnormality (range, 3–56%) in chronic eosinophilic leukaemia (CEL).[29] We should keep in mind that CEL, CEL-NOS, and idiopathic hypereosinophilic syndrome (HES) are not the same eosinophilic disorders. Formerly, WHO 2008 incorporated CEL/HES into HES under the MPN category, and also described 'CEL, not otherwise categorised' (CEL-NOC) and 'myeloid neoplasms associated with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* and *FGFR1*'.[30] In short, the latter group has been assigned a new category of its own, whereas both HES and CEL-NOC have remained subcategories of MPN according to WHO 2008.[30] WHO 2016 revised CEL-NOC as CEL-NOS, and HES and CEL could no longer be assigned as an MPN category. Moreover, many patients, who have been classified as idiopathic HES, can now be found to have a *FIP1L1-PDGFRA* fusion transcript.[31]

Table 3: Diagnostic criteria for CEL-NOS.[28]

- There is eosinophilia in blood (eosinophil count >1.5 x 10^9/L)
- There is no Ph chromosome or *BCR-ABL* fusion gene or other myeloproliferative neoplasms (PV, ET, PMF, systemic mastocytosis) or MDS/MPN (CMML or atypical CML)
- There is no t(5;12) or other rearrangement of *PDGFRB*
- There is no *FIP1L1-PDGFR-α* fusion gene or other rearrangement of *PDGFRA*
- There is no rearrangement of *FGFR1*
- The blast cell count in the peripheral blood and bone marrow is <20% and there is no inv(16)(p13q22) or t(16;16)(p13;q22) or other feature diagnostic of AML
- There is a clonal cytogenetic or molecular genetic abnormality, or blast cells are >2% in the peripheral blood or >5% in the bone marrow (but <20% to exclude acute leukaemia as a diagnosis)

AML: acute myeloid leukaemia; CEL-NOS: chronic eosinophilic leukaemia-not otherwise specified; CML: chronic myeloid leukaemia; CMML: chronic myelomonocytic leukaemia; ET: essential thrombocythaemia; FGFR1: fibroblast growth factor receptor 1; MDS/MPN: myelodysplastic/myeloproliferative neoplasms; PDGFRA: platelet-derived growth factor alpha; PDGFRB: platelet-derived growth factor beta; PMF: primary myelofibrosis; PV: polycythaemia vera.

Briefly, CEL and CEL-NOS are clonal diseases, whilst HES is not. CEL-NOS can be distinguished from CEL by the absence of *FIP1L1/PDGFRA* rearrangement. CEL-NOS can be distinguished from HES by the presence of a non-specific clonal cytogenetic abnormality or increased blast cells (>2% in the peripheral blood or >5% in the BM, but <20% blasts in both peripheral blood and BM). Furthermore, if *FIP1L1-PDGFRA* screening is not available, evaluation of the serum tryptase can be a useful surrogate marker for *FIP1L1-PDGFRA*-positive disease.[32] In the absence of *FIP1L1-PDGFRA*-positive disease, the cases of myeloid and lymphoid neoplasms with abnormalities of *PDGFRA*, *PDGFRB*, *FGFR1*, and *PCM1-JAK2* fusions should be excluded. Finally, absence of rearrangements of *FIP1L1/PDGFRA*, *PDGFRA*, *PDGFRB*, and *FGFR1*, and absence of *PCM1-JAK2* fusions should prompt consideration of a diagnosis of CEL-NOS when there is cytogenetic and/or morphologic evidence of an eosinophilic myeloid malignancy that is otherwise not classifiable.[33]

Cases with CEL-NOS are characterised by aggressive clinical course, and they are usually unresponsive to conventional chemotherapy.[34] The efficacy of currently used therapeutic agents is limited, and the haematological responses are usually short-lived. Similar to other MPN, hydroxyurea (1,000 mg/day starting dose) can be used to control leukocytosis and eosinophilia, but with no proven role in favourably altering the natural history of CEL-NOS. IFN-α (starting dose of 1 million units by subcutaneous injection three-times weekly) can produce haematologic and cytogenetic remissions in HES and CEL-NOS patients. The optimal dose and duration of hydroxyurea and IFN-α therapy is unknown and can be tailored to individual response and tolerability. Iurlo et al.[35] demonstrated the presence of a KIT^{M541L} allele variant in four out of five CEL-NOS patients who were negative for *PDGFRA* and *PDGFRB* abnormalities. They concluded that imatinib, which is effective in *FIP1L1-PDGFRA* rearrangement positive HES, was highly effective also in KIT^{M541L} variant cases.[35] Namely, imatinib may be considered for some selected CEL-NOS patients. Essentially, imatinib is recommended as a first-line therapy in patients with *FIP1L1/PDGFRA*-positive CEL.[36-38] 100 mg/day is sufficient to induce a complete molecular response in most patients with an *FIP1L1/PDGFRA* fusion gene. Nevertheless, the long-term prognosis, drug withdrawal, and drug resistance therapy remain unclear.

Acquired resistance to imatinib can occur because of point mutations in the ATP binding site (e.g. *T674I* and *D842V*).[39,40] *T674I* FIP1L1/PDGFRA mutations may be considered as analogous to the T315I mutation in *BCR/ABL1*, and the prognosis is poor for CEL patients harbouring T674I *FIP1L1/PDGFRA*. Recently, Jin et al.[41] have demonstrated that ponatinib is a potent inhibitor of CEL cells bearing wild-type or T674I *FIP1L1/PDGFRA*, that induces apoptosis in *FIP1LI/PDGFRA* expressing cells, making it a promising therapeutic prospect for the future.

The prognosis of CEL-NOS is poor. In a recently reported cohort of 10 patients, the median survival was 22.2 months, and 50% of the patients developed acute transformation to AML after a median of 20 months from diagnosis.[42] Three of the five patients who did not develop AML died with active disease; one patient underwent an ASCT and maintained a long-term remission, and the remaining patient achieved a complete remission on imatinib and hydroxyurea.[42] ASCT remains a curative option only for younger and fit patients with CEL-NOS.

MYELOPROLIFERATIVE NEOPLASM, UNCLASSIFIABLE

MPN-U is the least frequent subtype of MPN, and little is known about the incidence, clinical features, and management of this rare entity. MPN-U is considered when an MPN has definite MPN features; however, it does not meet diagnostic criteria for either the classic or the other non-classic MPN, or has features that overlap two or more of the MPN categories.[43]

In a study, *CALR* mutations were most frequent in patients with MPN-U (37.5%) compared with mutations in the PMF and ET groups (14.8% and 17.7%, respectively).[44] Most current data have also indicated that the CALR-mutated group may have a favourable prognosis compared with patients with *JAK2* mutations.[45,46] A previous study found that *CALR* mutations were associated with younger age, higher platelet count, lower thrombosis risk, and less leukocytosis, and were less likely to be transfusion dependent.[45]

MPN-U requires the absence of *BCR/ABL1*, dyserythropoiesis, disgranulopoiesis, or monocytosis, and the presence of effective clonal myeloproliferation leading to peripheral blood granulocytosis, thrombocytosis, or erythrocytosis. The presence of any one of dyserythropoiesis, disgranulopoiesis, or monocytosis mandates disease assignment to either the MDS or MDS/MPN category. The MDS/MPN are a unique group of myeloid malignancies characterised by both myelodysplastic and myeloproliferative features. MDS-like features include cytopenias and dysplasia of various cell lines while MPN-like features include constitutional symptoms, such as night sweats and weight loss, and elevated blood counts, as well as extramedullary infiltration.[47] Therefore, MPN-U should be distunguished from MDS/MPN-U.

In a recent study which is rather indicative of the general characteristics of the disease, 71 consecutive patients with a diagnosis of MPN-U were investigated.[48] They found that most of the cases displayed a hypercellular BM (70%) with normal erythropoiesis without left-shifting (59%), increased granulopoiesis with left-shifting (73%), and increased megakaryocytes with loose clustering (96%).[48] In this study, megakaryocytes displayed frequent giant forms with hyperlobulated or bulbous nuclei and/or other maturation defects; more than half of the cases displayed severe BM fibrosis (59%); median values of haemoglobin level and white blood cell counts were all within the normal range; in contrast, median platelet count and lactate dehydrogenase were increased. Of the patients, 44% showed splenomegaly. *JAK2* mutations were detected in 72% of all patients, and among the *JAK2* negative cases, *MPL* and *CALR* mutations were found in 17% and 67% of the cases, respectively.[48]

Currently used therapeutic agents for MPN-U are very limited, and a lack of data prevent us from making a clear recommendation. The very limited case reports from the literature demonstrate hydroxyurea improves blood counts and constitutional symptoms. In addition, the role of medications (except hydroxyurea and transplantation therapy) are not well established. We hope that the accumulating data will help us to understand the whole nature of this rare entity in the near future.

CONCLUSION

In conclusion, the non-classic MPN are rare clonal haematologic diseases, and an accurate diagnosis is mandatory for the management and predicting prognosis in this group of diseases. In this context, molecular genetic testing is the most important tool for establishing diagnosis. However, keep in mind that having a documented disease-related

mutation may help to establish an MPN diagnosis, but the absence of a specific mutation does not rule out MPN. The outcomes of current therapeutic approaches, as well as ASCT, are not satisfactory, and also there is no standard of care for CNL, CEL-NOS, and MPN-U. However, great efforts are being spent on discovery of new molecular pathways and development of molecularly targeted therapy. Therefore, participation in clinical trials likely to be conducted is especially encouraged for these extremely rare patient populations.

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NOVEL IMMUNOTHERAPY AGENTS FOR ACUTE LYMPHOBLASTIC LEUKAEMIA

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ABSTRACT

Acute lymphoblastic leukaemia (ALL) in adults has a survival rate of 40–50% at 5 years, with a high relapse rate after first-line chemotherapy. After relapse, results with salvage therapy are currently unsatisfactory. Therefore, both the optimisation of front-line therapy to reduce relapse incidence and the search for effective salvage therapies for relapsed/refractory (r/r) ALL have been of great interest to the medical community in recent years. The well-characterised expression of well-defined cell-surface antigens in B cell ALL (B)-ALL and T cell (T)-ALL, such as CD19, CD20, CD22, and CD52, has led to the development of several immunotherapy strategies, comprising 'nude' monoclonal antibodies (moAbs), conjugated moAbs, bispeciphic, or highly sophisticated chimeric antigen receptor (CAR)-T cell therapy.

Recently, both the bispecific moAb blinatumomab (anti-CD19 coupled with a CD3 recognition subunit) and the conjugated anti-CD22 moAb inotuzumab-ozogamicin have resulted in higher remission rates (44% versus 25%, and 80.7% versus 29.4%, respectively) and survival advantages (median overall survival [OS]: 7.7 months versus 4 months, and 7.7 months versus 6.7 months, respectively) in patients with r/r B-ALL when compared to standard salvage chemotherapy-based regimens. On the other hand, preliminary reports show feasibility and unprecedented response rates of ≤90% in highly refractory children and adults treated with CAR-modified T cells targeting the B cell specific CD19 antigen, which seem to be durable in a significant proportion of patients. Furthermore, the addition of anti-CD20 moAb rituximab to front-line standard chemotherapy in patients with CD20+ B-ALL has resulted in a clinical benefit, with prolongation of response duration and survival (3-year leukaemia-free survival and OS: 70% versus 38%; p<0.001, and 75% versus 47%; p=0.003).

In conclusion, immunotherapy is currently providing additional options for high-risk ALL patients both in front-line or advanced phase. Nonetheless, the optimal positioning of these novel agents, specially in relation to allogeneic haematopoietic stem-cell transplantion, needs to be clarified. This article aims to review several of these new therapeutic immunotherapy options available for patients with adult ALL, as well as their specific toxicity profile.

Keywords: Acute lymphoblastic leukaemia (ALL), immunotherapy, blinatumomab, inotuzumab-ozogamicin (IO), chimeric antigen receptor (CAR) T-cells, rituximab.

INTRODUCTION

Acute lymphoblastic leukaemia (ALL) is the most common childhood malignancy, with an incidence of 5 per 100,000.[1] In adults, ALL is less frequent, with an incidence of ~1 per 100,000.[2] Whereas the 5-year overall survival (OS) in children is ~90%, outcomes in adults are less favourable, at ~40%.[2,3] In adults, despite an initial high response rate of 80–90% with front-line treatments, a significant

proportion of patients experience a relapse. Applying paediatric-like regimens to adolescents and young adults optimises front-line therapy efficiency.[4] After a first relapse, the chance to obtain a subsequent complete remission (CR) with standard chemotherapy-based regimens ranges from 30–40%, and decreases to 10–20% after further relapses. Moreover, only a minority of these patients will be able to receive allogeneic haematopoietic stem cell transplantation (allo-SCT); this is the only treatment that allows durable responses in a significant proportion of relapsed ALL patients.[5-7]

In this context, there is a great interest in optimising front-line therapy to prevent relapse and find more effective salvage therapies for relapsed/refractory (r/r) ALL. The stable expression of several antigenic markers in B cell ALL (B-ALL) and T cell ALL (T-ALL), such as CD19, CD20, and CD22 cell-surface antigens, has led to the development of diverse antibodies with significant clinical activity directed against these antigens. Antibodies such as blinatumomab, inotuzumab-ozogamicin (IO), and rituximab have already been approved by the US Food and Drug Administration (FDA).[8-10] Recently, chimeric antigen receptor (CAR)-T cell therapy has shown promising results in treatment of r/r ALL, although it is still in the early stages of development.[11,12]

CD19-DIRECTED THERAPY

CD19 is a cell-surface receptor expressed on B cells from the late pro-B cell stage until plasma cell differentiation.[6,7] Blinatumomab, approved by the FDA (2014) and the European Medicines Agency (EMA) (2015) for the treatment of r/r philadelphia-negative (Ph-) B-ALL.

Blinatumomab is a bispecific T cell engager antibody, derived from a B-linage-specific mouse monoclonal antibody (moAB). Thus, one arm binds CD3 while the other binds CD19, redirecting unstimulated primary human T cells against CD19-positive lymphoma cells.[8,13] Activating proliferation of CD4+ and CD8+ cells and inducing granzyme and perforin-mediated serial tumour lysis upon recognition of CD19 antigen in B-cells, including B-ALL blasts.[3,5,6,14]

Blinatumomab has been administered in two different clinical settings with different tumour loads: in patients with persistent or reappearing minimal residual disease (MRD) and for patients in overt haematological relapse. Interestingly, in a small series of patients with positive MRD, blinatumomab was capable of inducing durable responses with MRD clearance in 16 out of 18 patients.[15] Furthermore, this agent showed a high clinical activity in r/r Ph- B-ALL. Thus, the response rate in a Phase II trial that included 189 patients with high-risk relapse was 43%, within two courses, including 33% CR. Forty percent of treated patients subsequently received an allo-SCT. Adverse events included two unexpected, blinatumomab-specific events, such as Grade 3 cytokine release syndrome (CRS) in 3 patients and Grade 3–4 neurological events in 20 patients, which included seizures, aphasia, and encephalopathy. This drug must be administered in continuous intravenous infusion over 4 weeks at a dose of 28 µg/day (9 µg/day during the first week).[15,16]

In a confirmatory Phase II study of 116 adult patients, 78% had a complete MRD response rate after one cycle of blinatumomab. The most frequent adverse events were tremor, aphasia, and encephalopathy.[17] In a Phase II study in r/r Philadelphia-positive B-ALL, 44 of 45 patients failed the second or later-generation tyrosine kinase inhibitor therapy, of which 16 (36%) patients achieved CR or CR with partial haematological recovery. The adverse events were those described previously.[18]

A recent, large, Phase III study has shown the superiority of blinatumomab compared to standard salvage chemotherapy in patients with refractory or high-risk (i.e. with a response duration <12 months or following allo-SCT) B-ALL r/r in terms of response rate (44% versus 25%) regarding CR and CR with incomplete haematological recovery and survival. Nonetheless, median OS after blinatumomab was 7.7 months, and blinatumomab is recommended as a 'bridge strategy' to bring patients to allo-SCT, although the overall result of such a strategy is currently unknown. Although the mechanisms of resistance to blinatumomab are incompletely elucidated, the emergence of a CD19 negative subclone can be observed in 30–50% of patients.[19]

CAR are recombinant antigen receptors with an anti-tumour target specificity, generated with the purpose to redirect autologous or allogeneic T lymphocytes or natural killer cells against tumour cells.[20] Diverse CAR T-cells have been engineered with different antigenic specificity and several costimulatory constructs to induce a durable response. Nonetheless, most clinical experiences accumulated correspond to treatment of advanced phase B-ALL and other B cell malignancies with

anti-CD19 CAR-T cells. CD19 is considered an adequate target for treatment of B cell malignancies, since it is a surface marker expressed during all B cell ontogeny and expressed in almost all B cell malignancies, including ALL.[20] Adoptive transfer of anti-CD19 CAR-T cells has resulted in an unexpectedly high rate of response in highly refractory B-ALL populations,[20,21] with CR rates of ≤90% and 1-year survival >50%, in both children and adults.

Experience with CTL019, anti-CD19 CAR with 4-1BB (CD137) costimulatory molecule (University of Pennsylvania, Pennsylvania, USA) in paediatric and adult B-ALL reported 27 of 30 patients (90%) were in a morphologic CR at the first assessment, 1 month after the infusion of CTL019. The rate of event-free survival at the median follow-up of 6 months was 67% in this heavily pretreated population.[20]

Davila et al.,[11] in a clinical trial involving 16 patients with r/r B-ALL who were treated with autologous T cells expressing the CAR 19-28z specific for the CD19 antigen, demonstrated that the response rate was 88%, allowing the transition of most of these patients to an allo-SCT as definitive therapy.[11] Lee et al.[22] recently reported a CR rate of 70% in a National Cancer Institute (NCI) analysis of 20 children and young adults with ALL.[22,23] Overall, there has been substantial experience with CAR therapy in children and adults with B-ALL, and response rates have not varied with age.

Treatments with CAR-T have been associated with unexpected toxicity, considered to be related to the rapid expansion of CAR-T clones, namely CRS and neurological dysfunction.[11] A few cases of CRS and neurotoxicity with fatal outcome have been described. Laboratory markers of systemic inflammation, including C-reactive protein and ferritin levels, were elevated in all the patients. Patients who had severe CRS had higher peak levels of interleukin (IL)-6 than patients who did not have severe CRS ($p<0.001$). Predictive factors and optimal management (treatment with tocilizumab/anti-IL-6 for severe CRS) resulted in a complete reversal of symptoms and a normalisation of laboratory results. Relapses ocurred in two of the nine patients who received immunosuppressive therapy for the CRS.[11,22]

Moreover, therapy with anti-CD19 CAR-T is followed by persistent B cell aplasia (on-target toxicity), which requires immunoglobulin reposition.[11,12,22,24-26] Randomised controlled trials comparing the efficacy of CD19-CAR versus blinatumomab r/r B-ALL are not available. CAR-based therapies have, on average, demonstrated higher remission rates than those reported with blinatumomab[24-26] A major distinction between blinatumomab and CD19-CAR-T cells is a difference in the duration of anti-leukaemic effects (CD19-CAR>blinatumomab).[24-27]

In summary, CD19-targeted CAR-T therapy is an emerging therapy that results in a high rate of response in both children and adult B-ALL refractory, compared to other therapeutic alternatives; responses that are durable in a significant proportion of patients. Although CAR-T cell therapies are still at an early stage, several observations suggest potential benefits. To date, the most dramatic results have been seen in ALL.[11,12,22]

A number of issues surrounding this therapy remain uncertain: extended follow-up is required to assess the proportion of patients that can be cured, the requirement of subsequent allo-SCT, optimal anti-CD19 CAR-T construct, the management of toxicity, predictive factors of response, and overall further development (CAR-T with dual targets, etc.). The worldwide availability of this therapy is also unclear.

ANTI-CD20 THERAPY

Rituximab is a chimeric moAb against CD20. Patients with B-lineage ALL may also have the CD20 antigen, being one of the first moAb that was evaluated as a treatment for patients with ALL.[28] Interest in this kind of condition is because ˜30–50% of precursor B cells express the CD20 antigen on the surface, which is targeted by rituximab. In addition, there are data suggesting that the expression of CD20 carries a worse prognosis in ALL.[29]

Maury et al.,[10] in a multicentre trial with adults with CD20-positive (CD20+) Ph- ALL randomised patients to receive chemotherapy with or without rituximab and demonstrated that patients assigned to the rituximab group had longer event-free survival than those assigned to the control group with GRAALL-2005 chemotherapy regimen (hazard ratio [HR]: 0.66; 95% confidence interval [CI]: 0.45–0.98; p=0.04). The estimated 2-year event-free survival rates were 65% (95% CI: 56–75) and 52% (95% CI: 43–63), respectively. The safety profile was quite good and the study showed that infectious events were slightly more frequent in the rituximab group, but the difference was not significant.

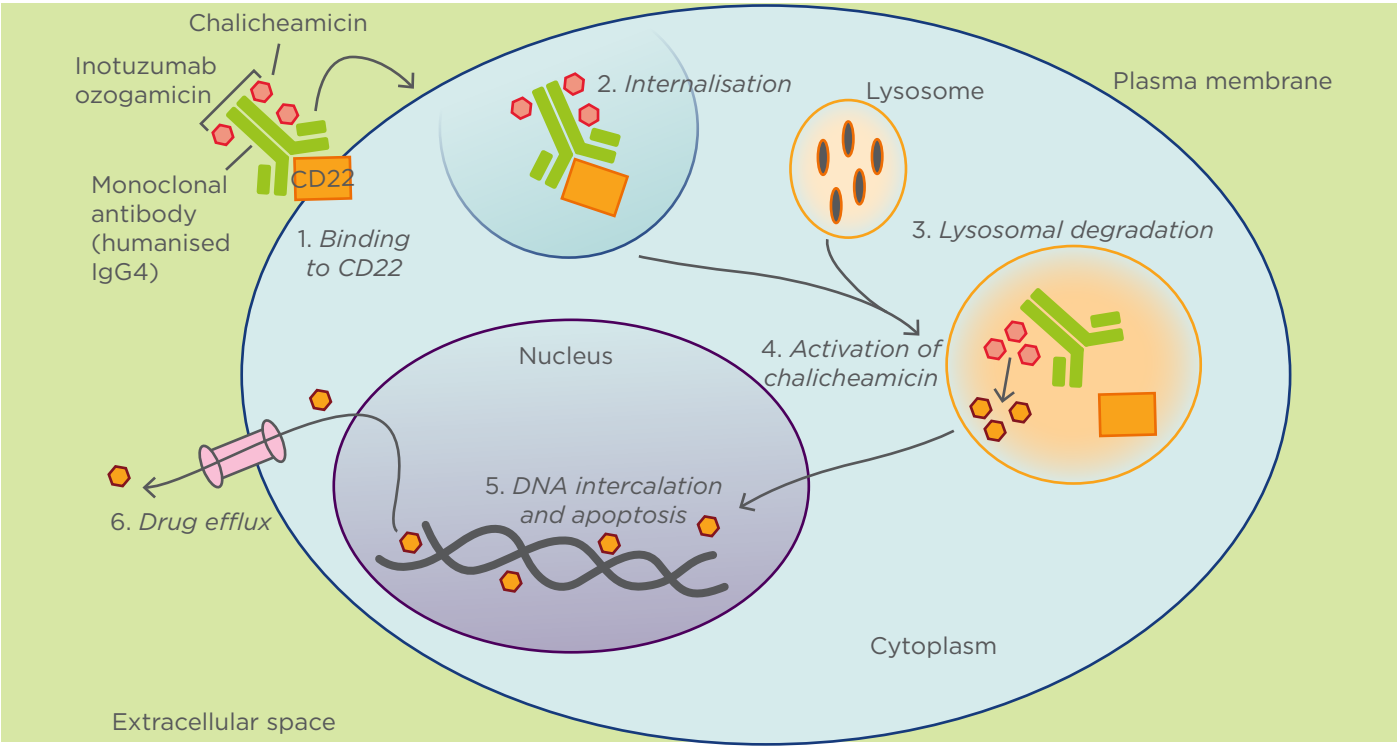

Figure 1: Proposed mechanism of action of inotuzumab ozogamicin.
IgG4: immunoglobulin G4.

Similarly, Thomas et al.[30] in a prospective, sequential, open-label, single-centre, Phase II trial, treated 282 adolescents and adults with newly diagnosed Ph- B-ALL with standard or modified hyper-CVAD regimens. The latter incorporated standard dose of rituximab when the expression of CD20 was >20%. The results suggested that the addition of rituximab to the hyper-CVAD-based regimens for the CD20+ precursor ALL-B significantly improved CR rates at 3 years (70% versus 38%; p<0.001) and OS (75% versus 47%; p=0.003).[30]

The German group was able to demonstrate improvement in the 3-year CR and OS rates (64% versus 58%; p=0.009; and 75% versus 54%, no p value given) when incorporating rituximab to standard chemotherapy in the context of the GMALL protocol 07/2003.[31,32] Studies to date have demonstrated the advantages of the association of rituximab in standard therapeutic protocols for patients with CD20+ Ph- ALL, with a safety profile quite similar to standard therapy.[10,33]

Recently, second-generation anti-CD20 moAbs, such as ofatumumab (Type I) and obinutuzumab (Type II), have been used in CD20+ B-ALL. Addition of ofatumumab to hyper-CVAD chemotherapy has been used in a Phase II trial with a limited number of patients with promising results, and is currently being used in an ongoing clinical trial in combination with BFM chemotherapy.[34-36]

CD22-DIRECTED THERAPY

CD22 is a B-lineage specific antigen expressed in >90% of leukaemic blasts in patients with ALL. It is a 135 kDa sialoglycoprotein that is expressed from the early to late stages of differentiation of B cells, with loss of expression in plasma cells.[37] CD22 is part of the immunoglobulin superfamily and what is interesting, from a pharmacological point of view, is that CD22 is rapidly internalised when binding to its antigen occurs, so it can be conjugated to a cytotoxic component to increase its efficacy (Figure 1).[38]

IO is a humanised moAb against CD22 conjugated to calicheamicin, a cytotoxic agent derived from *Micromonospora echinospora*.[39] Calicheamicin induces breaks in DNA double-strands and apoptosis independent of cell cycle progression.[40] IO is the most developed targeted antibody in this group. IO's ability to induce cell death is dose and time dependent and maximal saturation of CD22 is not essential for efficient cell death. The hypothesis

is that there is a continuous renewal of the cell surface expression of CD22 with an unstopped binding of IO and subsequent accumulation of calicheamicin inside the cell, which leads to apoptosis.[31]

A two-group, randomised, Phase III trial, assigned adult patients with CD22+ r/r ALL to receive either IO or standard intensive chemotherapy.[42] IO was given intravenously at a 1.8 mg/m^2 dose receiving 0.8 mg on Day 1 and 0.5 mg on Days 8 and 15, based on a Phase II clinical trial. The first cycle had a duration of 21 days and from the second cycle the duration was 28 days. The other group received chemotherapy of investigator's choice with three possible standard regimens.

Of the 326 patients who underwent randomisation, the first 218 (109 in each group) were included in the primary intention-to-treat analysis of complete remission. The primary endpoints were CR and OS. The results showed a CR of 80.7% versus 29.4% ($p<0.001$) and OS of 7.7 months versus 6.7 months (HR: 0.77; $p=0.04$). High remission rates were noticed in patients with both higher (>90%) and lower (<90%) levels of CD22 expression. Other results showed a CR with MRD below the threshold (0.01% marrow blasts) of 78.4% versus 28.1% ($p<0.001$), duration of remission of 4.6 months versus 3.1 months (HR: 0.55; $p=0.03$), and progression free survival 5 months versus 1.8 months (HR: 0.45; $p<0.001$). More patients proceeded to SCT after treatment in the IO group (41% versus 11%; $p<0.001$). It is noticeable that in patients with Ph+ ALL or t(4:11)+ ALL remission rates did not differ significantly between treatment groups.

Liver-related adverse events were more frequent in the IO group. They included increased levels of aspartate aminotransferase, alanine aminotransferase, and bilirubin. Veno-occlusive liver disease (VOD) of any grade occurred in 15 patients (11%) and cases were reported ≤2 years after randomisation. During, or shortly after treatment, five patients were diagnosed with VOD, two of which had undergone a SCT before randomisation. Of the 45 patients of the IO group who underwent a SCT after the trial, 10 patients had VOD, with the procedure being the second transplantation for 3 patients. The median time to the development of VOD after SCT was 16 days. Two treatment-related deaths due to VOD occurred after post-trial transplantation. In the standard chemotherapy group no cases of VOD were noticed during treatment and, of the 20 patients who underwent SCT, 1 patient was diagnosed with VOD.[42,43]

A study to identify prognostic factors in r/r ALL patients receiving IO was performed. By multivariate analysis, a high peripheral blood absolute blast count (>1x10^9/L) and low platelet count (<100x10^9/L) was associated with a lower chance to achieve bone marrow CR. Also, baseline features were seen to independently affect survival, including cytogenetics (complex karyotype, translocation [4;11], translocation [9;22], abnormal chromosome 7), disease beyond first salvage therapy, and high peripheral absolute blast count. Based on the three previous features, patients with 0, 1, 2, or 3 adverse factors had a median survival of ≥42, 8.8, 7.1, and 2.6 months, respectively.[42]

IO is also being tested as a first-line treatment in old patients given the high toxicity related to conventional chemotherapy. There is an ongoing Phase II clinical trial for patients ≥60 years of age with newly diagnosed ALL who receive a combination of low intensity chemotherapy with mini-hyper-CVD, IO, rituximab, and intrathecal chemotherapy.[38] Of the 20 patients included, with a median age of 69 years, an overall response rate of 95% has been observed, with 75% CR and 20% C-reactive protein. Progression free survival at 1-year has been 83% and OS 84%. Grade 3–4 non-haematological toxicities included increased liver enzymes and VOD in 1 patient; these are similar adverse effects to those observed in previous studies. These results suggest that combination of low intensity chemotherapy to targeted antibodies in first-line treatment of the elderly may obtain better outcomes than standard therapy.[44]

CONCLUSIONS

The development of immunotherapy will provide additional opportunities of treatment in r/r ALL and other haematological diseases. Rituximab combined with conventional chemotherapy improves the results in survival, and recurrence-free survival. CAR-T cell therapy against CD19 has obtained response rates ≤90% in some series. Blinatumomab and IO have achieved better response rates compared to conventional chemotherapy. Safety in these new drugs has been tested and adverse events are acceptable.

Certainly, much remains to be explored in the field of immunotherapy for patients with ALL. Use of these drugs as treatment is needed to verify results obtained in clinical trials and further studies are needed to examine long-term results. The next steps to be taken should be exploring the results of immunotherapy as first-line treatments (combined with standard chemotherapy or not) and finding new drugs to improve outcomes in T-ALL.

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A THEORETICAL VIEW OF OVARIAN CANCER RELAPSE

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ABSTRACT

Ovarian cancer (OC) is a disease that almost invariably relapses even after optimal primary cytoreductive surgery and standard first-line platinum-based chemotherapy. After recurrence, progressions occur at shorter intervals in the natural history of the disease. However, the biologic and cellular events underlying recurrence and progression (maintenance phase) are yet to be completely understood. Ovarian adenocarcinoma, like any other tissue, after reduction of the cell population (cytoreduction) either by surgery, chemotherapy, radiotherapy, or targeted therapies induced cell-death, tends to its own renewal through cancer stem cells (CSC). CSC remain quiescent most of their lives and then 'wake up', generating a proliferative progeny that differentiates as they become different clones of daughter cells. What defines them is their 'self-renewal' potential, thus perpetuating the disease with higher tumour volume relapses in which CSC increase in number.

We propose a theory of how recurrence/relapse occurs in which CSC play a key role in the genesis of relapse. These self-renewing CSC can generate a proliferative progeny and this population is sensitive to chemotherapy, anti-angiogenic agents, and PARP inhibitors, which so far have only increased the disease/relapse free survival ('maintenance phase'). In OC it seems we are not addressing the 'root' of recurrence/relapse. As with any theory, this is based on both proven facts and suggested hypotheses, which may serve as investigation drivers towards finally making a substantial improvement in OC management.

Keywords: Ovarian cancer (OC), relapsed ovarian cancer, cancer stem cells (CSC), maintenance therapy.

INTRODUCTION

Ovarian cancer (OC) is a disease of multiple relapses (Figure 1).[1] After achieving a complete response with first-line platinum-based chemotherapy, >70% of the patients recur at 2 years and subsequently, progression is almost unavoidable; >70% show progression at 1 year.[2-11] Many efforts have been made in order to improve these results: delivering chemotherapy intraperitoneally, in a dose dense schedule, or even adding a third agent to the standard carboplatin-paclitaxel backbone, which remains after >10 years. Antiangiogenic agents have also been incorporated after first-line treatment, but as with all the other attempts, only improvement in progression-free survival (PFS) with no impact on overall survival (OS) has been reached.

The same concept applies for treatments after relapse (maintenance therapies): the use of antiangiogenic agents (bevacizumab, cediranib, trebananib)[6,12-14] or PARP inhibitors, such as olaparib,[15] only delays progression, with PFS becoming shorter (Figure 1).

The objective of this publication is to provide a theoretical view of OC relapses, focussing on their biological genesis, and to envision potential targets that may provide a substantial benefit in OC patient management. For this we performed a PubMed search, restricted to full-text articles, using the following search terms: 'ovarian cancer', 'cancer stem cells', 'relapsed ovarian cancer', and 'ovarian cancer maintenance'.

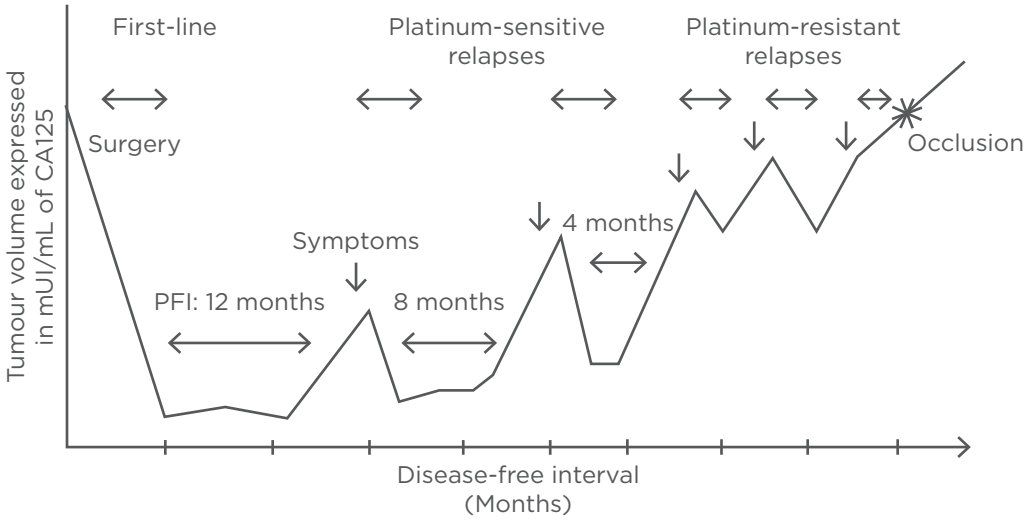

Figure 1: Natural history of ovarian cancer evolution.[1]
PFI: platinum-free interval.

BIOLOGY OF RELAPSE

As stated before, recurrence seems to be an unavoidable event in OC history since >70% recur even after being optimally debulked (R0) at first cytoreductive surgery and standard platinum-based chemotherapy. These recurrences/progressions can occur at varying intervals and have been classified according to platinum-free interval (PFI), the interval in months since the last platinum-dose, into the following categories:

- Platinum-refractory/resistant: PFI: <6 months
- Platinum-partially sensitive: PFI: 6–12 months
- Platinum-sensitive: PFI: >12 months

This classification was derived from retrospective data from Phase III randomised trials showing that efficacy of platinum-based chemotherapy was directly proportional to the PFI in terms of overall response rate (ORR), PFS, and OS.[16,17] This distinction reflects that OC is an heterogeneous disease with different aggressive biology phenotypes, and also phenotypically, with mucinous and clear cells having a worse outcome than their more frequent and chemo-sensitive counterparts high-grade papillary serous and endometroid subtypes.[18]

Nowadays, we know that microscopic disease that survives first-line chemotherapy and second or third (at relapse) is responsible for recurrent disease or progressive disease, yet responds to the same chemotherapy agent (platinum) with ORR ≥70%, especially in *BRCA* mutated patients.[19] In the homologous-recombination deficient (HRd) population (which is estimated to be 50% of the patients),[20] the use of maintenance therapy with olaparib increased PFS by 9.4 months, but with no impact on OS.[11] Coincident with these results, Phase III trials using antiangiogenic agents (bevacizumab, cediranib, trebananib) as maintenance therapy after platinum-sensitive or refractory relapse only prolonged ORR and PFS by targeting angiogenesis concurrent to and after chemotherapy.[10,13,14,21,22] Although these different agents have proven to be effective in delaying progression, this still occurs at shorter intervals. Many unresolved issues remain about the biology of this disease diagnosed at relapse. Table 1 summarises some unanswered questions about relapse.

Table 1: Unsolved issues about cancer stem cells.

Unsolved issues about CSC in OC
1) Why does the disease relapse at different intervals with worse prognosis? Is it chemo-resistant micro-metastatic disease?
2) If so, why is the ORR 70–80% in second and third-line therapy? (with exactly the same combination as first-line therapy)
3) What are the driver-events that lead to CSC stemness or awakeness?
4) Why does targeting angiogenesis or DNA repair mechanisms in proliferating progeny derived from CSC only increase PFS?
5) Is platinum-resistant disease richer in CSC?

CSC: cancer stem cells; OC: ovarian cancer; ORR: overall response rate; PFS: progression-free survival.

THE CANCER STEM CELL THEORY

We know that tumours are heterogeneous not only in their histopathology, but for a certain type of tumours, like high-grade papillary serous, in the function of cells that comprise them, their proliferative capacity, as well as responsiveness to different agents (chemotherapy or antiangiogenics).

The CSC theory proposes that within a tumour there is a certain hierarchy with CSC being responsible for recapitulating the disease after cytoreduction generated by surgery, chemotherapy, or both. These CSC, which represent only a small portion of the tumour (<1%), a proportion that increases at relapse (self-renewal capacity), remain quiescent in the G0 phase of the cell cycle and therefore, not vulnerable to chemotherapy agents which damage DNA or microtubules in the S or M phase of the cell cycle. They are tumourigenic (able to create tumour foci when injected in immunosuppressed mice),[23-26] clonogenic (able to create a proliferating, differentiating progeny),[27,28] and pluripotent (not only able to generate tumour cells but microenvironmental cells as well).[29] It is their proliferating progeny that are vulnerable to chemotherapy agents, especially because OC cells are deficient in repairing chemotherapy-damaged DNA.[30,31]

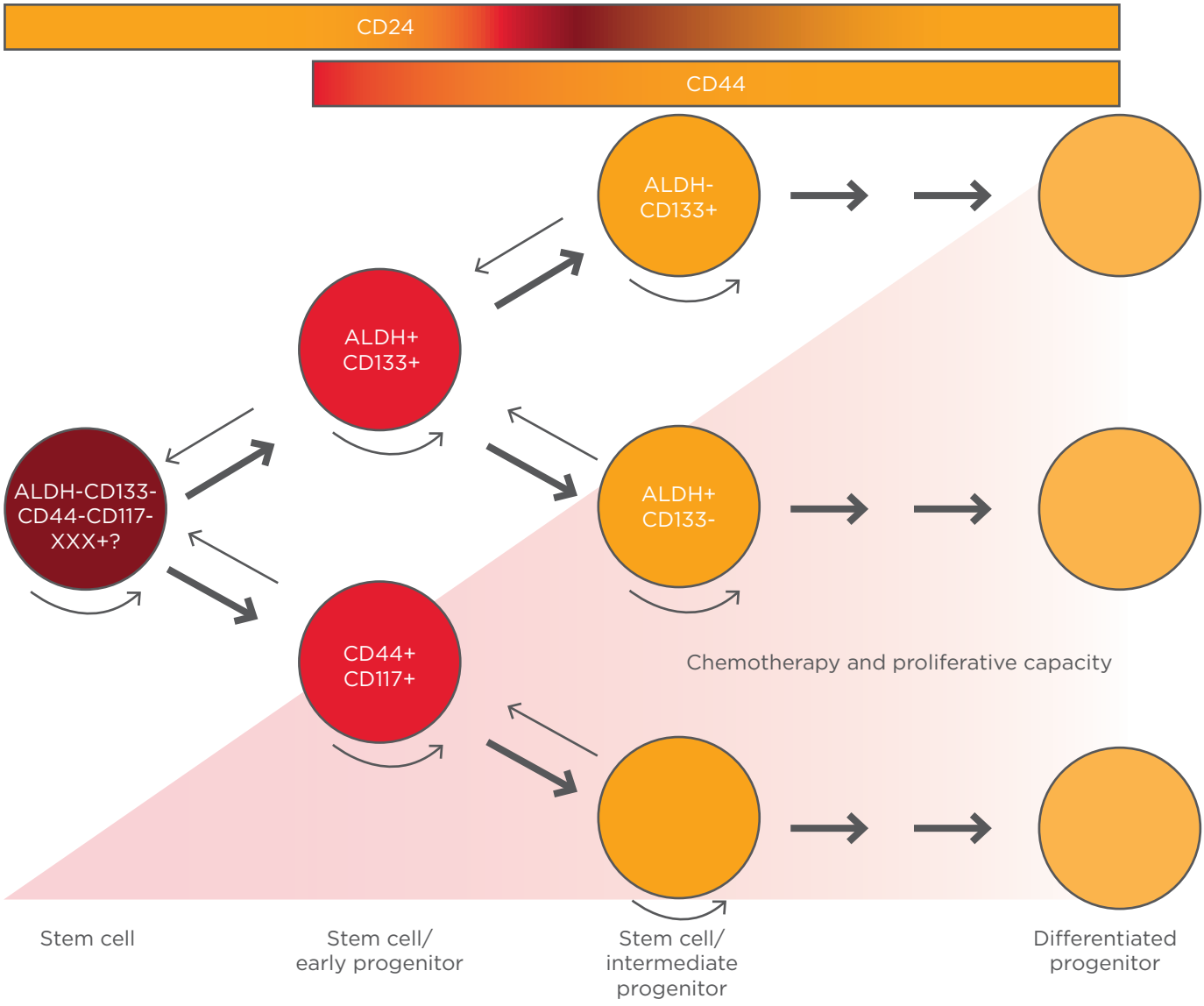

Figure 2: Hierarchy in ovarian cancer and cancer stem cell differentiation.
Rounded arrows represent self-renewal capacity. Small arrows indicate the unproven potential for dedifferentiation.
ALDH: aldehyde dehydrogenase.
Adapted from Burgos-Ojeda et al.[31]

Thus, according to this theory, OC may be regarded as an aberrant tissue that arises from CSC, which in turn may have arisen from normal stem cells (SCs) after a mutation that generated this self-renewal phenotype, or from a mutation in a normal cell that allowed it to dedifferentiate into a CSC or early progenitor.[32] Hence, they create a 'back-up' population that is able to recapitulate the disease. This self-renewal capacity is what defines this progeny with a 'SC-like phenotype'.

CSC are a subpopulation of cells within the tumour that have different markers of chemo and radio-resistance (CD133, aldehyde dehydrogenase, CD44) as well as CD24 (related to tumour-initiating capacity).[31] They have the capacity to repopulate foci of tumour at sites of primary undebulked disease after achieving complete response due to chemotherapy, but can also metastasise early to distant microscopic foci, generating OC relapse/recurrences.[20,33] Changes in the microenvironment or any other 'driver-event' or 'transforming-event' (endocrine, paracrine, or autocrine factors) wake them up from that quiescent state and allow them to proliferate asymmetrically: one daughter-cell repopulates the CSC pool and the other starts to proliferate, generating a progeny with different pathways of differentiation (some cells can differentiate to endothelial cells depending on the stimulus that the microenvironment provides) or even dedifferentiate to a more 'SC' phenotype.[34] Figure 2 shows CSC differentiation and tumour-cell hierarchy in OC based on the current literature.

CSC act as an insurance population and are responsible for relapses and progressions in OC in which CSC population is higher.[35] There is clinical evidence to support this CSC hypothesis in OC, and it has also been extensively studied in other tumour models, such as breast, colon, and leukaemia.[36-40]

CLINICAL EVIDENCE TO SUPPORT THE CANCER STEM CELL THEORY IN OVARIAN CANCER

Although not extensively studied in terms of their impact on OC prognosis, some evidence suggests that CSC correlate with a high volume of disease at recurrence and a worse prognosis.[31] To date, the only therapeutic manoeuvre with a positive impact on OS is macroscopically removing all foci of tumour (R0)[41] harbouring CSC, in other words rendering the patients optimally debulked, or R0, at the time of primary cytoreductive surgery. Nevertheless, clinical evidence suggests that microscopic foci of disease also harbour CSC generating recurrent/progressive disease.[33]

High Response Rates at Relapse with Same Chemotherapy Regimens

As stated earlier, the longer the disease-free survival (DFS)/PFS, the better the results with platinum based chemotherapy. If recurrence/relapses were generated by surviving chemo-resistant clones, why does the platinum-sensitive relapse have an ORR >70% with the same chemotherapy combination?[42]

The high ORR are due to the effect that chemotherapy has on a proliferating progeny with a frequent mutation in p53 (a critical checkpoint for cell-cycle progression) and a HRd phenotype in 50% of the patients.[43] *p53*, *BRCA1,* and *BRCA2* mutations (the hallmark of HRd) are described as 'early driver-events' in OC carcinogenesis. Is the platinum-refractory/resistant disease richer in CSC or is chemo-resistant chemotherapy an early progenitor?

Trials with Antiangiogenic Agents as Maintenance Therapies

Two pivotal Phase III trials (GOG 218 and ICON 7)[6-12] with bevacizumab and one with pazopanib (AGO-OVA),[44] after first-line chemotherapy, demonstrated a benefit in DFS for patients treated with bevacizumab, especially in high-risk groups: suboptimally debulked and Stage IV.

In the relapse setting, these agents have been evaluated as well: the OCEANS trial for platinum-sensitive relapse and AURELIA for platinum-resistant relapse. In both, the addition of bevacizumab to chemotherapy not only improved ORR but also PFS. In the 'platinum-resistant relapse', the patients who benefited the most were those who presented with ascites, in whom bevacizumab improved global and disease-specific quality of life scores.[10,21,22] ICON 6 evaluated cediranib,[13] a vascular endothelial growth factor (VEGF) tyrosine-kinase inhibitor, as maintenance after platinum-sensitive relapse. The experimental arm that received cediranib as maintenance had a significant improvement in PFS (hazard ratio [HR]: 0.56; 95% confidence interval [CI]: 0.44–0.72).

It is well known that the tumoural microenvironment changes after chemotherapy, with haemorrhagic necrosis in sites of chemotherapy-induced response. This implies an improvement in hypoxic conditions. This microenvironmental improvement may be a

'driver event' for CSC to abandon their quiescent state and regenerate the tumour, because conditions are now more favourable.[45] However, clinical evidence shows that targeting the VEGF pathway, either through bevacizumab or cediranib, does not create a situation of chronic hypoxia for CSC to remain quiescent and the proliferating progeny finds alternative ways of angiogenesis or survival under these hypoxic conditions,[46,47] as patients who relapse after getting bevacizumab first-line do not derive more benefit by adding it to chemotherapy at relapse.[48]

PARP Inhibitors in Maintenance Trials

PARP inhibitors are the first types of agents approved in OC based on a predictive biomarker for response (*BRCA1* and *BRCA2* mutations).

This subgroup of OC patients, which represents 14% of cases considering germline-mutation positive (*gBRCA*^{m}) but ≤20% if somatic-mutation (*sBRCA*^{m}) patients are added,[43] have the HRd phenotype, meaning that the most faithful, error-free, DNA damage-repair mechanism for DNA double strand damage is inefficient. This kind of damage is caused by carboplatin: double strand DNA-adducts, which can only be efficiently repaired by homologous recombination. Many factors interact with this mechanism, but *BRCA1* and *BRCA2* play a key role. The mutations of the genes that encode for these nuclear proteins are the most prevalent mutations leading to this HRd phenotype.[49] Other genes involved in homologous recombination are less frequently mutated and lead to a 'BRCAness or BRCA-like' phenotype (HRd phenotype). These types of tumours are less capable of repairing the damage chemotherapy induces in their DNA, and, consequently, are more chemo-sensitive. In fact, *BRCA*^{m} OC has a better prognosis than *BRCA* wild-type (*BRCA*^{wt}).[50]

PARP inhibitors create a situation in which OC cells are forced to use this HRd mechanism. By trapping PARP, a key factor in one of the mechanisms for repairing single-strand DNA breaks, like the one reactive species of oxygen created in the hypoxic tumour microenvironment, they create a situation of 'synthetic lethality': single-strand DNA breaks become double-strand breaks as the replication fork gets stalled inside the double helix by the PARP inhibitors. These agents take advantage of this cell deficiency and create a genomic instability state, making the cell utilise an alternative method of double-strand DNA damage repair; non-homologous end joining in which essentially, the injured DNA is excised and the proximal and distant ends joined, producing extensive loss of genetic material, which is critical for cell survival.[51,52]

Study 19 is a Phase II trial that evaluated the activity of olaparib, the most extensively studied PARP inhibitor in OC, as maintenance therapy after platinum-sensitive relapse (a surrogate of HRd). They demonstrated a benefit in PFS for the global population (HR: 0.65; 95% CI: 0.25–0.49), but the difference was greater among *BRCA1* and *BRCA2* mutated patients (germline or somatic) who received maintenance with olaparib: (HR: 0.18; 95% CI: 0.11–0.31). This agent is the first target agent to show such a PFS benefit with a low toxicity profile (12% patients on treatment at 5 years), given orally. Nevertheless, no benefit in OS was demonstrated for the olaparib treated patients, not even the *BRCA*^{m}.[11]

This suggests that even though we can target a defective mechanism of utmost importance for cell-survival (DNA repair), the HRd proliferative progeny either develops mechanisms of resistance or alternative repair mechanisms to survive despite these mutations.

Combination of Antiangiogenic Agents and PARP Inhibitors

Liu et al.[53] presented the data of a Phase II trial in which patients with platinum-sensitive relapse were randomised either to olaparib or a combination of olaparib plus cediranib. This trial showed that the combination favoured *BRCA*^{wt} population in terms of PFS over *BRCA*^{m} patients. The reason behind this finding is the concept of 'contextual synthetic lethality' in which cediranib-induced hypoxia downregulates replication, transcription, and transduction of factors involved in homologous recombination, making the *BRCA*^{wt} (homologous recombination 'efficient') behave as HRd.[54] This combination is being evaluated against a platinum-based combination and olaparib in platinum-sensitive relapse as a chemotherapy-free regimen, and the efficacy will very likely be the same. However, this combination did not significantly improve OS, even targeting two mechanisms that are part of the biology underlying relapse/progression (angiogenesis and DNA damage repair response). Therefore, unless we target the real source of relapse: CSC, OC will still be considered a disease of multiple relapses.

CANCER STEM CELLS AS A TARGET

One of the most important hurdles to clear in targeting CSC is the lack of knowledge of their biology: why or what makes them remain in this quiescent state that renders them less vulnerable to chemotherapy or DNA damaging therapies. It seems that hypoxic conditions lead to the expression of a 'SC-like' phenotype as a way of the tumour ensuring its perpetuity. What are the 'driver-events' that lead them to enter in cell-cycle, apart from improvement in tumour micro environment? Is it a paracrine factor or even an endocrine factor whose systemic concentrations are low when tumour-burden is low after chemotherapy or surgery induced cytoreduction?

It has been demonstrated that the Hedgehog, Notch, and Wnt pathways are key signalling pathways for CSC differentiation and apoptosis.[55] Through these mechanisms CSC develop the whole differentiation 'programme' (plasticity). Other histological subtypes, which account for less than a third of OCs, probably are driven by a different type of differentiation either by genetic or environmental factors. The rare types: clear cell, mucinous, endometroid, and low-grade adenocarcinoma, have their own driver mutations (*PTEN*, *PI3K/AKT*, *ARID1A*, *Her2/neu*, *BRAF*, *KRAS*)[56-58] which explain some of their biological behaviours, and also possess new targets for the maintenance phase after chemotherapy (in which no chemotherapy regimen other than standard carboplatin/paclitaxel has been demonstrated to be better in first-line therapy, regardless of the subtype). In this range of subtypes, low-malignant potential tumours (borderline tumours) might represent the most differentiated form. In fact, Emmanuel et al.[59] demonstrated in 14 micro-dissected specimens with borderline and invasive tumours coexisting in the same sample that chromosome copy number aberrations were remarkably conserved in the invasive and borderline components, suggesting that the latter could be the 'fully-expressed differentiation programme version' of the invasive form. Is there a way to promote this differentiation of the CSC generated progeny?

CSC express markers like OCT4A, NANOG, and SOX2. These proteins are fundamental for differentiation and survival. T cell reactivity against OCT4A has been demonstrated, making it targetable for vaccination and inducing an anti-CSC memory immune response. Ongoing trials are evaluating dendritic cell vaccination against these CSC antigens.[60]

CONCLUSIONS

Although efforts have been made to improve the results of first-line chemotherapy, as well as treatment at relapse, only improvements in DFS/PFS have been accomplished without a dramatic impact on OS. Over the last 30 years not much has changed in OS at 5 years with <30% of patients remaining alive.

The biology and the underlying cellular and molecular events that take place during the maintenance phase (variable periods between relapses) are far from being completely understood. Targeting two of the known mechanisms that lead to progression, angiogenesis, and DNA damage repair mechanisms, have shown similar results (increased PFS, no benefit in OS). Unless the root of progression: CSCs or tumour initiating cells are targeted, OC will still be a chronic disease with multiple relapses.

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