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FOCUS ISSUE

Hematology

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Journal of Interdisciplinary Medicine

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The Journal of Interdisciplinary Medicine aims to publish top quality papers related to any fields of medicine that present an interdisciplinary dimension.

The journal will mainly focus on recent advances in the field of diagnosis and treatment of the most common situations encountered in the clinical or research practice. Interdisciplinary approaches will be extremely welcomed, presenting new advances in the approach of different pathologies from the perspective of various clinical fields.

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EDITORIAL

Epicardial Fat-mediated Inflammation: a Major Player in Cardiovascular Diseases

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Hematological disorders are involved in a large number of systemic diseases in which complex interdisciplinary approaches are required to ensure the most effective treatment strategy. Stem cell therapy has emerged as a promising therapeutic tool, not only in patients with hematological disorders, but also in patients with acute myocardial infarction, severe heart failure, critical limb ischemia, neurological disorders, or for cartilage regeneration in various orthopedic applications.[1,2] Modern bioengineering technologies made it possible to generate scaffolds impregnated with stem cells for various applications (such as patches for wound repair or for open heart surgery).[3,4] It has also been demonstrated that in patients with heart failure or with coronary artery disease, correction of anemia can lead to a significant improvement of symptoms and outcomes.[5,6]

Several hematological parameters carry also prognostic impact for other diseases. For instance, interventional cardiology studies demonstrated that neutrophil count, leucocyte count, or blood count-derived parameters such as red cell distribution width-platelet ratio (a new biomarker for inflammation) or neutrophil-lymphocyte ratio are significant predictors for the occurrence of no-reflow phenomenon after successful recanalization of an occluded coronary artery in the setting of an acute myocardial infarction.[7–11]

Therefore, hematology can be considered as an important link between various diseases, serving also as a reflection of the severity of these pathologies. This special issue of JIM is focused on the most relevant topics in modern hematology.

Bzduch *et al*. performed a study on the importance of FLT3-ITD gene mutation in the survival of patients with acute myeloid leukemia (AML). The authors analyzed 210 patients with AML, from which 10 presented FLT3-ITD mutation, and the results showed an overall survival rate of 7% (n = 15), while patients with the specified mutation presented a 100% mortality rate (n = 10) within the first two months. Moreover, none of the subjects with FLT3-ITD mutation presented complete remission of the disease.[12]

Stem cell therapies have been used for the treatment of hematologic neoplastic diseases for over three decades, including Hodgkin and non-Hodgkin limphomas.[13] Pakucs *et al*. aimed to evaluate complications occurring after au-

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tologous hematopoietic stem cell transplantation on 94 patients with malignant lymphomas. The analysis revealed that 18% of subjects presented confirmed infections, 42% presented electrolyte imbalances, and 36% had hypoalbuminemia.[14] The results of this study underline the importance of a proper evaluation of complications that are related to stem cell therapies, in order to implement proper patient management.

Furthermore, Mild *et al.* performed a retrospective analysis on patients with failure of stem cell mobilization, in which the highest harvesting failure occurred in patients aged between 51 to 61 years, and all the analyzed subjects had been heavily pretreated with alkylating agents and purine analogs. Moreover, the study included patients with various types of hematological malignancies, and the results found that the highest mobilization failure occurred in non-Hodgkin lymphoma (42%, n = 8), Hodgkin lymphoma (37.5%, n = 6), and multiple myeloma (21%, n = 4).[15] Being that autologous stem cell therapies can be a curative option for patients with various hematological cancers, further studies on the cause and factors associated with failure to harvest stem cells, as well as methods for improvement are essential.

Located at the border between hematology and cardiology, thrombosis and cardioembolic events in atrial fibrillation patients have been linked to increased systemic inflammation, hypercoagulable states, and enhanced atrial fibrosis.[16,17] Oltean *et al.* are currently conducting a clinical observational study on atrial fibrillation patients, the FIBROS study, in which they will test the hypothesis that the degree of atrial fibrosis, in correlation with several hematological markers for inflammation, platelet aggregation, and clotting, could serve as a model for identifying the profile of patients at increased risk for cardioembolic events.[18]

Due to the versatile use of stem cells in various therapies, there has been an increasing interest in cellular applications, not only in hematological disorders, but also in non-healing ulcers, as well as in different types of peripheral artery disease.

This focus issue includes a case report on the effect of stem cell transplantation that led to improvement of both the hematological malignancy for which it was intended, but it also caused a significant improvement in the overall cardiac function. More specifically, Tudor *et al.* presented a patient with multiple myeloma, who went into the remission phase after stem cell transplantation, and presented an increase in the left ventricular ejection fraction from 50 to 60%, a smaller mitral regurgitation jet, and also a lower systolic pressure in the pulmonary artery.[19] The success of stem cell treatment on both the hematological condition as well as the associated cardiovascular condition is indicative of the pluripotent capacity of these novel treatments in complex patients with multiple comorbidities.

In an article published in this focus issue by Opincariu *et al.*, the authors sought to review the current articles on the use of stem cell treatments, not only on subjects with critical lower limb ischemia, but also on several types of peripheral artery disease, caused by either atherosclerosis or thromboangiitis obliterans, including upper limb ischemia, stroke patients, renal artery stenosis, and mesenteric ischemia.[20] The manuscript revealed the presence of extensive research on lower limb ischemia, which has shown promising results in restoring blood flow to no-option ischemic limbs, in which revascularization methods (surgical or interventional) either had failed, or were not suitable.[21] At the same time, stem cells have been proven effective in improving motor deficits in ischemic stroke patients, but the main concern in these procedures is safety, the type of stem cell to be used, as well as the route of injection. Preclinical studies on swine and rat models are also cited by Opincariu *et al.* in stem cell therapeutic applications for renal artery stenosis and mesenteric ischemia, both showing promising results in the improvement of renal fibrosis and glomerular filtration rates, as well as increased survival and better recovery of the intestinal barrier after induced intestinal ischemia.[22,23]

Another manuscript in this issue is concerning the use of cell therapies in non-healing ulcers, published by Mester *et al.* The authors present a clinical update on the use of various types of stem cells, harvested from the bone marrow, adipose tissue, umbilical cord, and placenta, that present paracrine effects, trigger angiogenesis, cell proliferation, and tissue regeneration, thus inducing ulcer healing and epithelization, while simultaneously decreasing the local fibrosis.[24] There is multiple evidence regarding the efficacy of stem cells in the improvement of various types of non-healing wounds caused by skin cancer, irradiation, diabetes, and ischemia, leading to the belief that stem cells could become a first line of treatment in chronic ulcers that present no tendency for regeneration.[25–28]

All three manuscripts present possible prospects in stem cell therapies in hematological diseases, complex cardiovascular disorders, peripheral artery disease, and non-healing wounds, describing the emerging use of allogeneic cells from matched HLA healthy donors, as well as the ethical and financial limitations of these methods. However, all authors mention the great therapeutic potential of stem cells in disorders in which other treatment methods have failed.

Novel therapies in various diseases that include stem cell transplantation require a proper collaboration between a multitude of specialties. Stem cell harvesting and mobilization for their use in cardiac and vascular disorders are performed with the help several specialists including the hematologist. Various cardiovascular diseases are influenced by hematological parameters, while hematological disorders and their treatment can have great impact on the cardiovascular balance, thus underlying once again the importance of a proper collaboration between specialties for both a proper patient management, as well as in preclinical and clinical research.

CONFLICT OF INTEREST

Nothing to declare.

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REVIEW

CARDIOLOGY // HEMATOLOGY

Stem Cell Therapies in Peripheral Vascular Diseases — Current Status

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ABSTRACT

Peripheral artery diseases include all arterial diseases with the exception of coronary and aortic involvement, more specifically diseases of the extracranial carotids, upper limb arteries, mesenteric and renal vessels, and last but not least, lower limb arteries. Mononuclear stem cells, harvested from various sites (bone marrow, peripheral blood, mesenchymal cells, adipose-derived stem cells) have been studied as a treatment option for alleviating symptoms in peripheral artery disease, as potential stimulators for therapeutic angiogenesis, thus improving vascularization of the ischemic tissue. The aim of this manuscript was to review current medical literature on a novel treatment method — cell therapy, in patients with various peripheral vascular diseases, including carotid, renal, mesenteric artery disease, thromboangiitis obliterans, as well as upper and lower limb artery disease.

Keywords: stem cells, peripheral vascular diseases, endothelial precursor cells, therapeutic angiogenesis

INTRODUCTION

According to the 2017 guideline of the European Society of Cardiology on the management of peripheral artery disease (PAD), the term "PAD" includes all arterial diseases with the exception of coronary and aortic involvement, more specifically diseases of the extracranial carotids, upper limb arteries, mesenteric and renal vessels, and last but not least, lower limb arteries.[1] Peripheral arterial disorders affect approximately 40 million inhabitants in Europe, leading to increased healthcare costs, as well as high morbidity and mortality rates and impaired health-related quality of life.[2–5] The most frequently cited cause for PADs with various locations is atherosclerosis, therefore the risk increases with age and exposure to classical cardiovascular risk factors including dyslipidemia, diabetes, chronic tobacco use, arterial hypertension, obesity, and newer factors such as enhanced systemic inflammation, hyperhomocysteinemia and various genotypes.[6] Ongoing research is required for developing new therapeutic measures for subjects that present no indication for either inter-

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ventional or surgical revascularization — the so called "no-option" patients.

The aim of this manuscript was to review current medical literature on a novel treatment method — cell therapy, in patients with various peripheral vascular diseases, including carotid, renal, mesenteric artery disease, thromboangiitis obliterans, as well as upper and lower limb artery disease.

PERIPHERAL ARTERY DISEASES — MORE THAN WORDS CAN SAY

As previously mentioned, PADs are located in several vascular sites, leading to devastating repercussions that comprise multiple aspects of the healthcare system from an economical point of view, as well as individual quality of life, morbidities and mortality rates.

Patients with PAD present increased risk for cardiovascular adverse events irrespective of the site of the lesions. Subjects with carotid artery disease have been shown to have increased risk of acute cerebrovascular events, acute myocardial infarction, and cardiac death.[7] The prevalence of asymptomatic carotid stenoses of >50% is 4.2%, showing an increasing rate with age in Europe,[8] while in the United States, the rate of moderate to severe carotid artery disease was found to be 3.9%.[9]

Upper extremity ischemic disease is less common than lower limb PAD, accounting for less than 5% of all cases of limb ischemia.[10] Atherosclerotic disease is rarely the sole culprit of critical ischemia of the upper limbs; more often, Buerger's thromboangiitis obliterans or systemic sclerosis are the responsible causes.[11–13] Subclavian stenosis is frequently caused by atherosclerotic plaques, with an increasing prevalence from 2% in the general population, to 9% in subjects with lower limb PAD.[14] The clinical presentation of subclavian artery disease includes various symptoms, from hand claudication to several manifestations of the subclavian steal phenomenon, with cerebral hypoperfusion, or, in subjects with coronary bypass grafting and axillo-bifemural bypass, it could lead to chest pain or lower limb claudication respectively.[15,16]

Thromboangiitis obliterans, or Buerger's disease is a non-atherosclerotic segmental inflammatory disease characterized by the presence of thrombosis in the small and medium arterial vasculature, affecting mainly young subjects with a positive history of chronic tobacco use, with frequent distal upper limb involvement, but it can also affect the lower limbs, leading to severe ischemic pain, gangrene of the extremities, and culminating with limb amputation.[17]

Mesenteric artery disease is often undiagnosed in clinical practice, and it causes approximately 5% of all acute intestinal ischemic events.[13] The coeliac trunk is more often affected than the superior mesenteric artery, as it was shown by a study on a population undergoing cardiac catheterization, in which the prevalence of mesenteric artery disease was 14%, out of which 11% was located in the coeliac trunk.[18]

Atherosclerotic renal artery disease has been shown to affect 6.8% of subjects in the Cardiovascular Health Study, regardless of gender or age, although it affected significantly more male patients than females (9.1% versus 5.5% respectively, $p = 0.05$), and its incidence was independently associated with increasing age, LDL cholesterol levels, and increasing systolic blood pressure.[19] Another study on 866 patients undergoing simultaneous coronary and renal artery angiography, found significant atherosclerotic renal artery stenosis in 39.8% of cases, from which 22.3% were with bilateral involvement, while age, female gender, hypertension, left anterior descendant and circumflex artery stenosis of more than 50% were found to be independent predictors for renal artery stenosis.[20]

Approximately 202 million subjects suffer from lower limb PAD around the globe, showing an increasing incidence with age. The gender distribution varies between lower and middle-income states, where women are more affected than men, and higher income countries, where there is a net male predominance in the non-elderly population.[21] Critical limb ischemia, the end stage of chronic lower limb PAD, is present in 500–1000 new cases per million, with increasing incidence among diabetic patients, and is not only associated with high morbidity and mortality rates, but also with high risk of limb amputation.[22–25] The annual amputation rate above and below the knee is between 120 to 500 in every million lower limb PAD subjects.[26] The death rate related directly to lower limb PAD is 3.5 per 100,000 individuals, but most patients will succumb to complications related to coronary artery disease or stroke, as PAD is a marker for systemic atherosclerotic involvement.[13,27]

THERAPEUTIC USE OF STEM CELLS IN PERIPHERAL ARTERY DISEASES

Bone marrow-derived stem cells (BMSC) have been studied as a therapeutic option for alleviating symptoms in PAD as potential stimulators for therapeutic angiogenesis, thus improving vascularization of the ischemic tissue and enhancing both perfusion and woundhealing.[28] Cell therapies with either BMSC or progenitor cells derived from

peripheral blood may offer ongoing sources of growth factors and structural tissue components for vessel regeneration or neoformation.[29]

Angiogenesis refers to the development of preexisting capillary endothelial tubules as a response to tissue ischemia, being mediated by hypoxia-induced release of vascular growth factors and related citokines.[30] Arteriogenesis is the development of the collateral vascular network by an increase in the diameter of the preexistent collateral arterioles, which will act as a sustaining vascular network that compensates the function of the occluded vessels.[31–33] The physiological stimulation of arteriogenesis occurs in conditions of increased shear stress, leading to a mechanical increase in vessel diameter, followed by the activation of adhesion molecules and cytokine release that will attract circulating monocytes, which are actually bone-marrow derived cells. Monocytes activate matrix proteases that will create the spatial conditions needed for vessel growth, and within 3–4 weeks from the occlusion of a large artery, the collateral arteries will be able to provide a proper blood flow to the affected tissue.[34,35] Several studies have sought to find the role of the bone marrow derived monocytes in the arteriogenesis process, and the findings suggest that the stem cells stimulate artery development not by incorporating into the vessel wall, but by promoting cytokine release, which offer paracrine stimulation of vascular growth.[34,36]

Circulating endothelial progenitor cells (EPC) were first described by Asahara *et al.*[37] and were shown to originate from bone marrow-derived monocytes that are present in the vicinity of the collateral vessels.[38] Another observation that sustains the promoter role of BMSC in arterial collateralization present in PAD, was that the number of circulating monocytic endothelial progenitor cells was lower if risk factors such as diabetes, tobacco use, dyslipidemia, or old age were present. These are the same risk factors associated with failed collateral development and with the severity of PAD.[28,39–43]

These observations have led to the birth of a new concept: therapeutic stimulation of angiogenesis by stem cell infusion. Cell therapy aims to stimulate physiological arteriogenesis, by using an increased number of precursor cells that will provide the required cytokines for an accelerated arteriogenesis.[44] Several studies on cell therapies for improvement of tissue perfusion have been carried out, with either bone-marrow derived monocytes or peripheral blood mononuclear cells that express surface markers that identify human EPC, more specifically CD133, CD34, KDR (kinase insert domain receptor), and VEGF receptor 2.[45–48]

STEM CELL THERAPIES IN VARIOUS TYPES OF PAD

Carotid artery disease and stroke

Although cell therapies in stroke patients do no induce collateral vessel formation, there are several preclinical and clinical studies that have researched the effect of intravenous and intra-arterial stem cell injection for improving the neurological deficits of acute ischemic stroke patients.[49–53] Preclinical studies have found that the implantation of bone marrow mononuclear cells, which include, among other types, hematopoietic and mesenchymal stem cells, may reduce the size of cerebral infarction and improve the functional outcomes by producing various cytokines and growth factors.[54–57] These observations have set the base for multiple clinical studies in which stem cells were injected intracerebrally, intravenously, or intra-arterially in patients with acute cerebral ischemia, and all have found potential benefits in cell therapies for stroke patients.[58,59] However, several questions should be answered by larger randomized controlled clinical trials, regarding the timing (acute or chronic phase of brain ischemia),[60,61] the type of cell (bone marrow-derived, peripheral blood-derived, fetal cells),[49] route of delivery (intracerebral, intra-arterial, intravenous),[62–65] and infusion rates (larger vessels mean increased infusion rates and vice versa),[66,67] and last but not least, those regarding the safety of the procedure.[58,61,68,69]

Upper limb ischemia

While there is an increasing number of studies that sustain the clinical benefits of cell therapies in coronary artery disease and lower limb ischemia,[70–75] there are scarce data on their role in upper limb ischemia, which is rarer, but if present, is associated with significantly worse outcomes and increased mortality rates.[76,77] There is a whole body of evidence on the beneficial effects of different cell therapies in lower limb PAD,[78] but there are few data on the effect of angiogenesis stimulation in upper limb ischemia. However, there are reports of several cases with critical ischemia of the upper limb that have benefited from BMSC as an angiogenic inducer.[79,80] Camerota *et al.* have reported the case of a 63-year-old diabetic male with bilateral upper limb digital gangrene caused by atherosclerosis. The patient had received several injections at the level of the forearms and hands, in which bone marrow-derived tissue repair cells were delivered with the purpose of improving tissue perfusion. The 1-year follow-up showed a significant improvement in clinical perfusion sings (wound heal-

ing, no need for analgesics, complete resolution of pain, and improved quality of life), as well as a better perfusion, objectively seen with pletismography.[79] Nevskaya *et al.* reported 2 cases with ischemic digital wounds caused by systemic sclerosis, in which the patients had received mononuclear cells derived from the peripheral blood and from the bone marrow. The results showed increased skin perfusion, wound healing, and improved blood flow in the brachial artery in both cases.[81] A study on 7 subjects with hand ischemia (rest pain, non-healing ischemic ulcers), either caused by thromboangiitis or an autoimmune disease, found that at 6 months after the injection of a mixture of CD34+ and CD 133+ cells, all patients presented improved digital-brachial pressure index, lower score on the visual analogue scale for pain, and ulcer healing.[80] These reports and pilot studies may be suggesting that autologous bone marrow-derived cells could become a safe method for therapeutic angiogenesis in patients with critical hand ischemia as well; however, further research on larger patient populations is required.

Thromboangiitis obliterans

The role of cell therapies in improving ischemic signs and symptoms of patients with Buerger's disease has been studied in various clinical trials.[83–87] Subjects with Buerger's disease are not suitable candidates for revascularization procedures in the presence of critical limb ischemia due to the frequent involvement of distal low-caliber arteries. Lee *et al.* have performed intramuscular implantation of whole bone marrow stem cells in 90 limbs from 67 subjects with symptomatic Buerger's disease and observed significant clinical and angiographical improvement, as well as a decrease in amputation rates.[82] Kim *et al.* carried out a study on 27 patients with lower limb thromboangiitis who were not suitable for surgical or interventional revascularization, in which they implanted isolated EPCs from the bone marrow in the tibial bone, in association with subcutaneous injection of granulocyte colony-stimulating factor (GCS-F). During a mean follow-up of 19.1 months, from 17 limbs with non-healing ulcers, 13 were healed, and 14 patients presented visible collateral growth on the control angiography, while only 6 showed no collaterals. They also found a significant increase in the number of EPCs in the peripheral blood after GCS-F administration.[87] Motukuru *et al.* conducted a 6-month follow-up study on non-reconstructible Buerger's disease patients who underwent BMSC transplantation into the calf muscles of the affected limb. After 6 months, patients presented significant improvement in ulcer healing, increased ankle-brachial index ($p < 0.01$), and transcutaneous oximetry values ($p < 0.01$).[88] Similar results were found by Durdu *et al.*, who injected autologous BMSC after erythrocyte depletion, in various regions with ischemic lesions (gastrocnemius muscle, intermetatarsal region, feet dorsum, or forearm) in 28 patients with Buerger's disease, which were followed up for 16.6 ± 7.8 months. They observed that 83% of patients presented ulcer healing, all patients presented relief of rest pain and no need for analgesics, while in 78.5% of cases, collateral vessels had formed after 6 months from BMSC implantation.[89] The results of all these studies suggest the positive effects of angiogenesis stimulation with endothelial precursor cells in patients with Buerger's disease, in which established therapies such as smoking cessation or vasodilator therapies have failed, and who, due to the involvement of small-caliber vessels, are not candidates for surgical or interventional revascularization procedures. Thus, there is supporting data regarding the initiation of larger clinical trials for the assessment of cell therapies in subjects with thromboangiitis obliterans.

Mesenteric artery ischemia and reperfusion injury

Mesenteric ischemia is rarely caused by atherosclerosis (5% of intestinal ischemia cases),[13] being frequently undiagnosed. Intestinal ischemia is more often caused by necrotizing enterocolitis, trauma, septic shock, strangulated hernias and volvulus, or cardiac surgery, and it presents a very high risk of death.[89,90] No clinical research on cell therapies in mesenteric atherosclerosis have been conducted, but there are several preclinical studies on the effect of stem cells in intestinal ischemic and reperfusion injuries.[91–93] Jiang *et al.* investigated the effect of BMSC in intestinal ischemia on 100 rats in which the superior mesenteric artery was clamped for 45 minutes, followed by the injection of BMSC in the submucosa of the small intestine, followed by reperfusion.[93] Their results showed a significant reduction and an accelerated recovery of the intestinal barrier dysfunction in the BMSC group. Jensen *et al.* performed a similar study on a mouse population, in which human adipose-derived stromal cells were infused into the peritoneum, after 60 minutes of clamping the superior mesenteric artery. The results showed a higher 7-day survival, increased mesenteric perfusion, lower inflammatory status, and preserved intestinal architecture for adipose-derived stromal cell-treated mice.[91]

Stem cells have been shown to provide benefits in the case of intestinal ischemia and reperfusion injuries in preclinical settings, albeit no data is available on cell therapies in atherosclerotic mesenteric artery stenosis. These early

observations could represent a stepping stone for future research on the currently developing applications of stem cell therapies.

Renal ischemia

Atherosclerosis of the renal arteries is one of the major causes of chronic renal disease, which implies the gradual reduction of the glomerular filtration rate (GFR) due to ischemic loss of renal parenchyma, which will eventually lead to 6–27% of end-stage renal insufficiencies.[94,95] Restoring the impaired blood flow to the kidney in renal artery stenosis does not always succeed in reestablishing kidney function and improving the GFR; the identification of other restorative therapeutic measures is of utmost importance.[96] Mesenchymal stem cell therapies for various types of kidney injuries have been the subject of research in many preclinical and clinical studies, most of them focusing on acute kidney injury.[97–100] The repair mechanism of mesenchymal stem cells (MSC) includes cytokine release and MSC differentiation into renal cells; MSC can be implanted intravenously, intra-arterially, or within the kidney parenchyma.[101]

Ischemic chronic kidney disease caused by renal artery stenosis is characterized by glomerular fibrosis and decreased number of microvessels, with secondary triggering of the renin angiotensin aldosterone cascade, with subsequent vasoconstriction, inflammation and fibrosis.[101] Preclinical studies on swine models have proven the efficacy of cell therapies in chronic ischemic kidney disease, in which the intrarenal administration of endothelial progenitor cells has led to tissue repair, decreased inflammation and fibrosis.[102,103] Others have shown that the implantation of MSC in the renal artery, with or without concomitant revascularization, led to an important improvement of kidney function, as well as a decrease in oxidative stress, inflammation, and fibrosis.[104,105]

Saad *et al.* conducted a clinical study in which 14 subjects with atherosclerotic renovascular disease received an intra-arterial infusion of MSC in association to the standard medical treatment. These subjects were matched by 14 patients who had received medical treatment only. During the 3-month follow-up, the MSC group presented increased cortical perfusion, the renal blood flow rose in the stenotic kidney from 151.8 mL/min to 185.5 mL/min ($p = 0.01$), and kidney hypoxia decreased from 12.1% to 6.8% ($p = 0.04$), as assessed by blood oxygen level-dependent MRI.[106]

The current preclinical and smaller clinical studies on cell therapies in renal artery stenosis and its subsequent damage show that there is hope in restoring renal function in these patients, in which percutaneous revascularization alone is most often not a viable solution for repairing neither the kidney lesions, nor their local and systemic cardiovascular impact; however, further human studies are required for the clinical implementation of the procedure.

Lower limb ischemia

The rationale for cell therapies in lower limb artery disease is to promote collateralization and angiogenesis, and therefore to improve tissue perfusion and wound healing, and prevent amputations.[107] Cell therapies in lower limb ischemia are generally reserved for patients who do not present indication for interventional or surgical revascularization, or in whom these methods have failed, more specifically, for no-option critical limb ischemic patients.[108,109] There is extensive clinical research on the topic of therapeutic angiogenesis in lower limb PAD, showing a clear benefit in various types of precursor cells, either autologous (endothelial precursor cells, BMSC, peripheral blood stem cells, mesenchymal or adipose-derived stem cells) or allogeneic, the latter presenting the disadvantage of possible immune rejection.[110]

Several clinical trials have been conducted on bone-marrow derived mononuclear cells implantation in ischemic lower limbs. The TACT trial (Therapeutic Angiogenesis by Cell Transplantation), one of the first clinical studies on the matter, included a pilot study in which 22 patients with lower limb PAD had received BMSC in one leg and saline solution in the other, and a randomized clinical trial in which other 22 patients with ischemic lower limbs received BMSC in one leg and peripheral blood-derived precursor cells in the other. The results showed that in both studies there were significant improvements in transcutaneous oxygen pressure, pain-free walking time, and ankle brachial index, but the results were better in the patients treated with bone marrow-derived stem cells.[111] Other trials have also confirmed the efficacy of cell therapies in promoting vessel collateralization, symptom improvement, and wound healing in lower limb critical ischemia.[112–116] A meta-analysis published by Rigato *et al.* included 67 studies (randomized and non-randomized clinical trials, and non-controlled studies), on a total number of 2,352 patients with intractable lower limb PAD or critical limb ischemia, and the primary outcome of the studies was limb amputation.[112] From the analysis of randomized clinical trials, their results showed a 37% reduction on amputation rates after cell therapies, as well as a 59% improvement of wound healing, significantly lower rest pain and higher

ankle-brachial index and tissue oxygen pressure. They also found that intramuscular implantation of cells is more effective compared to the intra-arterial approach, and that bone marrow-derived mononuclear cells are better than peripheral blood cells or mesenchymal stem cells in obtaining successful results. Also, they found no important adverse reactions to cell therapies.[112]

Therapeutic angiogenesis in no-option critical lower limb ischemia is a promising novel treatment method that offers hope of escaping amputation, an improvement in the overall quality of life, pain-free time, and higher values for indicators of tissue perfusion (ankle-brachial index, tissue oxygen perfusion). The extensive preclinical and clinical data on autologous stem cell implantation, either in the muscle or in the artery, or a combination of the two, has the possibility to change the outcome of intractable critical limb ischemia.

THE FUTURE OF STEM CELL THERAPIES IN PAD

Well-planned randomized controlled studies are still needed to assess the long-term effects of stem cell implantation for different peripheral artery diseases. The use of programmed allogeneic progenitor cells in patients who present autologous stem cell exhaustion could present a new branch in the therapeutic angiogenesis tree, and the recruitment of stem cells from healthy, HLA-matched donors may provide alternative cellular sources that are less affected by chronic associated diseases.[117–119] Moreover, genetic therapies based on in vivo gene transferring for inducing angio- and arteriogenesis in combination with stem cell transplantation could better the outcome of patients in which all the applied therapeutic measure have failed.[107,120]

CONCLUSIONS

There are still multiple untouched territories in stem cell research, including their use in therapeutic angiogenesis for various peripheral vascular diseases. However, their efficacy in enhancing tissue oxygenation, angio- and arteriogenesis in the ischemic segment, as well as accelerating wound healing and improving the functional features of the affected organ, has been proved by various researchers in all peripheral artery diseases (carotid, renal, mesenteric, upper and lower limb). Despite the increasing body of data from preclinical studies for renal or mesenteric artery disease, as well as more frequent clinical trials for lower limb ischemia, there is a long road ahead until stem cell implantation will be listed in the therapeutic guideline recommendations for such disorders, requiring further multicenter randomized clinical trials on larger populations.

CONFLICT OF INTEREST

Nothing to declare.

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CLINICAL UPDATE

CARDIOLOGY // HEMATOLOGY

Stem Cell Therapy in Wound Healing

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ABSTRACT

Wound healing is a complex restorative process of the altered cutaneous tissue, which is impaired by numerous local and systemic factors, leading to chronic non-healing lesions with few efficient therapeutic options. Stem cells possess the capacity to differentiate into various types of cell lines. Furthermore, stem cells are able to secrete cytokines and growth factors, modulating inflammation and ultimately leading to angiogenesis, fibrogenesis, and epithelization. Because of their paracrine activity, these cells are able to attract other cell types to the base of the wound, improving the formation of new skin layers. Mesenchymal stem cells derived from the adipose tissue, bone marrow, and placenta, offer numerous ways of implementation. The process of harvesting, growing, and administrating stem cells depends on the site and type of the cells, but recent trial results showed improvement of wound healing independent of the administration site. Bioengineered skin substitutes are validated for treatment of chronic wounds with direct application on the skin surface. These offer physical scaffolding for the migrating cells and promote secretion of growth factors, thus facilitating rapid wound healing. Obtaining further clinical data is essential, but stem cell therapy may become a first-line therapeutic choice for the treatment of non-healing chronic wounds.

Keywords: stem cell therapies, non-healing wounds, bioengineered skin

INTRODUCTION

Chronic impaired wound healing is still a challenging therapeutic task for healthcare experts. It involves a vast interdisciplinary approach from surgical, medical, dermatological, diabetic, general practitioner specialist, and nursing staff for long-term success. Aging and various medical conditions create a physiopathological ground for impaired wound healing, leading to a long and exhausting restorative process, which often remains unsuccessful and greatly affects the quality of life of these patients.[1,2] As the aging population is growing worldwide, it is expected that the burden of chronic wounds will increase significantly in the next few decades.[3] Approximately 1% of the population suffers from non-healing wounds, generating tremendous costs for healthcare systems.[4,5] Despite the multitude of recently developed therapeutic options for these patients, the results are still unsatisfactory. Multiple clinical and preclinical trials are under development worldwide for the assessment of novel therapeutic methods in chronic wound healing.[6,7]

As a result of translational medicine research progresses, stem cells have emerged as a viable alternative for the treatment of this complex pathology for enhancement of tissue regeneration.[8] Stem cell therapy has already been intensely studied in organ damage repair and regeneration in numerous diseases.[9,10] One of the most promising results was obtained in the field of cardiovascular diseases, namely recovery in the post-myocardial infarction phase and critical limb ischemia.[11–13] The major drawback of stem cell-based treatment is represented by the difficulties of adequate stem cell population selection, delivery, and the prevention of immune and tumor responses.[14]

The aim of this clinical update is to summarize the current applications of stem cell therapies in the treatment of chronic wounds.

PATHOPHYSIOLOGY OF IMPAIRED WOUND HEALING

Regeneration and healing of the altered cutaneous tissue is a complex, well-organized process. After platelet aggregation and clot formation in the hemostasis phase, the inflammatory process is activated by chemokines, which attract neutrophils, lymphocytes, and monocytes that release inflammatory cytokines and growth factors.[15] Chronic wounds exhibit a prolongation of the inflammatory phase, which ultimately leads to failure of the healing process due to alteration of the extracellular matrix.[16] The proliferative and resolutive phase of wound healing is characterized by angio- and fibrogenesis, with collagen formation and re-epithelization. Chronic wounds are usually defined by deficient vascularization, with reduction of angiogenesis and formation of fibrotic and epithelial cells.[17] All stages can be altered by aging and by the presence of local factors, such as infections, ischemia, radiation, traumas, toxins, as well as systemic factors such as diabetes, cancers, drugs, neuropathies, smoking, or alcoholism.[18]

THE ROLE OF STEM CELLS IN WOUND HEALING

Given their pluripotent and self-renewing characteristics, stem cells are able to differentiate into different types of mature functional tissues.[19] Their potential in the epithelial healing of chronic wounds lies in their capacity to secrete cytokines that promote angiogenesis, cell proliferation, and tissue regeneration. The development of bioengineered tissues impregnated with stem cells stays at the basis of this novel chronic wound care therapy.[20] One of the most important factors that affect the success of stem cell therapy is represented by the selection of stem cell populations.

Mesenchymal stem cells

Mesenchymal stem cells (MSC) can be isolated from various types of tissues including bone marrow, umbilical cord blood, and adipose tissue, and possess the ability of good adherence to plastic materials.[21] These stem cells can be delivered topically or systemically.[22] In addition to their ability to differentiate into various cell lines and to promote angiogenesis, MSCs present potent immunomodulatory and immunoregulatory characteristics, thus coordinating the inflammatory process, reducing scarring, and modulating fibrosis. This is achieved by releasing multiple chemokines that act as mediators.[23,24] The efficiency of MSCs in the treatment of critical limb ischemia has already been proven safe and efficient.[25] Currently there is no evidence of any clinical benefit regarding the tissue origins of stem cells used in wound healing.

Adipose stem cells

Adipose stem cells (ASC) are pluripotent MSCs, which can be extracted from adipose tissue, are easily harvested by liposuction or minimal-invasive surgical excision and can be preserved for up to six months. Another advantage of adipose tissue over other extraction sites of MSCs comes from the fact that a large number of stem cells can be yielded from this type of tissue compared to other sites (e.g., 40-fold higher compared to bone marrow) and can be transplanted to autologous or allogeneic hosts.[26] Autologous ASC transplantation is well tolerated and it is proven to be safe in terms of malignant transformation.[27] Given the rich amount of easily accessible fat tissue in the body and their angiogenic and paracrine potential, ASCs are widely used MSCs in wound healing research.[28,29] ASCs improve wound healing by the secretion of mediators such as platelet derived growth factor (PDFG), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), fibronectin, and collagen I, stimulating angiogenesis and smaller scar formation.[30] Because of their paracrine effect, ASCs stimulate other cells and recruit stem cells to form fibroblasts, keratocytes, and endothelial cells, and down-regulate the inflammatory response, therefore leading to improved epithelization.[18,31] Numerous studies have analyzed the effect of ASCs in different types of chronic wound healing, with positive histological and clinical results in skin cancer-related wounds.[32] Ischemic and irradiation wounds are also an important topic in chronic wound

healing, with different results so far.33Another broadly researched application of ACSs is represented by diabetic wounds. Recent preclinical studies suggest that ASCs combined with artificial skin enhances diabetic wound healing through the secretion of growth factors and new vessel formation in diabetic rats. An improvement was observed in capillary formation, fibroblast proliferation, epithelization, and wound contraction compared to control groups.[34–36] Encouraged by the positive preclinical results, many ongoing clinical trials are being carried out, but few results have been published so far about wound healing. However, the results of other applications of ASCs (e.g., osteogenic defect healing and Crohn's disease fistulas) are promising.[37,38]

Bone marrow stem cells

Bone marrow is one of the most frequently used sites for stem cell extraction. These cells play an important role in each phase of wound healing, thus they are targeted as viable therapeutic alternatives in chronic wound healing.[39] Bone marrow-derived stem cells also hold the property to adhere to plastic materials and can be applied directly on the surface of the wounds, injected in the edges of the wound, or administered systemically.[40] Randomized controlled clinical trials have reported positive outcomes with topical and intramuscular delivery of bone marrow-derived MSCs and autologous biograft in chronic diabetic wounds. Significant contraction in wound size (7.26 ± 1.41 cm^2 vs. 2 ± 0.98 cm^2, $p < 0.001$) was observed at 12 weeks, with prolonged pain-free walking distance (38.33 ± 17.68 m vs. 284 ± 212 m, $p < 0.001$).[41] Another study, which used bone marrow-derived stem cells applied with a polymer spray on surgical excision chronic wounds, has demonstrated the efficiency of this therapy, leading to the repair of the epithelial surface in eight weeks.[42] An enhancement of the vascularity and growing of the dermal layer was observed in a study in which the researchers had injected bone marrow-derived stem cells in the edges of the wound in patients with diabetic foot. This was accompanied by a reduction in wound size.[43] Autologous bone marrow-derived stem cells have led to complete wound healing in a study that included patients with leg ulcers that lasted longer than one year despite conventional treatment.[44] In a randomized controlled trial where intramuscular bone marrow-derived stem cell were administered for diabetic patients with critical limb ischemia and foot ulcers, there was a significantly higher healing rate and pain-free walking distance recorded during the 24-week follow-up period.[45]

Placental and embryonic tissue

Placental tissue is an excellent source of stem cells, with pluripotent differentiation and immunomodulatory capacity, which makes it a suitable alternative for the bioengineering of skin substitutes that can be used for the treatment of chronic wounds.[46] Furthermore, growth factors and extracellular matrix that are essential for the natural wound healing process are also secreted by the placenta.[47] Preclinical investigations are carried out to determine the safety and efficiency of this method. Bioengineered skin substitutes are available from the human neonatal fibroblast-dermis, which promotes cell migration and, through a paracrine effect and secretion of growth factors, enhances epithelization. It also offers physical scaffolding for migrating cells into the base of the wound, helping the recovery and healing of chronic ulcers. Randomized trials have proved the efficacy of these skin substitutes in chronic diabetic ulcers, with significantly higher wound closure rates compared to controls at 12 weeks (30% vs. 18.3%, $p = 0.02$).[48,49] Another bioengineered skin substitute is derived from neonatal foreskin, and it is approved for clinical use, being based on a bilayer structure of fibroblasts and keratocytes that facilitates the migration of monocytes to the wound base and the secretion of growth factors such as PDGF, fibroblast growth factor (FGF), or granulocyte colony-growth factor.[50] These factors enable cell integration and allow vascular ingrowth, thus increasing and accelerating chronic wound healing. The published results of recent clinical trials are encouraging and have proved the efficacy of this treatment option in patients with diabetic foot ulcers and venous leg ulcers.[51,52] A median of 61 days was recorded until complete wound healing vs. 181 days in the control group ($p = 0.003$) in patients with venous leg ulcer.[53]

PROSPECTS OF STEM CELLS IN WOUND HEALING

The origin of stem cells, the process of harvesting, preservation, and manipulation of cell lines, as well as their use is still under strict regulations both in Europe and in the United States, and they raise ethical controversies in terms of research and clinical use.[54] Another limitation of stem cell therapy is linked to the huge costs of manufacturing, resourcing, and preservation of cell lines. Promising results of current stem cell therapies are encouraging investments in this flourishing research field, which leads to the emergence of new preclinical and clinical trials, which may transform it into a widely accessible therapeutic method.

CONCLUSIONS

Despite many current therapeutic options, chronic wounds still remain a great concern for many healthcare professionals, and the number of these patients is expected to grow in the future due to the aging population and increasing incidence of diabetes. Stem cell therapy holds the potential of clinical improvement for these patients, due to its capacity of tissue regeneration, and direct and paracrine activity. A considerable number of stem cell types are used in preclinical and clinical studies with encouraging results, but further trials are needed to validate this therapeutic option.

CONFLICT OF INTEREST

Nothing to declare.

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CLINICAL UPDATE

HEMATOLOGY // INTERNAL MEDICINE

Watch and Wait – Actualities in the Treatment of Chronic Lymphocytic Leukemia

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ABSTRACT

In Western countries, chronic lymphocytic leukemia (CLL) is one of the most diagnosed leukemia types among elderly patients. CLL is described as an indolent lymphoproliferative disorder, characterized by the presence of a high number of small, mature B-cells in the peripheral blood smear, with a particular immunophenotype (CD5, CD19, CD23 positive and CD20 dim positive) and accumulation in the bone marrow and lymphoid tissue (e.g., lymph nodes, spleen). The experience of the past decades showed that CLL is clinically very heterogeneous; while some patients present a chronic clinical evolution, with a prolonged survival, in which the treatment can be delayed, others suffer from a more aggressive form, which must be treated early and is associated with many relapses. This observation led to several genomic studies that have mapped the genetic modifications involved in the disease conformations, including del(13q14), del(11q), or trisomy 12. On the other hand, certain genetic mutations such as del(17p13)–p53, NOTCH1 mutation, or ZAP70/CD38 increased expression are associated with worse clinical outcome. In order to apply the right treatment strategy, the RAI and BINET staging systems should be considered, which are based on clinical and laboratory assessment, on genetic mutations that may influence the resistance to chemotherapy, as well as the patient's age and comorbidities. The aim of this manuscript was to present the therapeutic approaches of CLL, in order to attempt to answer the following question: to treat, or not to treat? This clinical update focuses on the managements of CLL patients in the 21st century.

Keywords: chronic lymphocytic leukemia, treatment, stem cell transplantation, conservatory therapy

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a frequent form of leukemic malignancy among adults in developed countries. The incidence was reported around 4 to 6 cases per 100,000 persons per year in Europe and in the United States. The incidence increases with age, most of the patients being diagnosed after the age of 65. A lower incidence of CLL is maintained in Asian individuals, however.

TABLE 1. The RAI staging system

Stage	Characteristics	Median survival
0	lymphocytosis (>5 × 10^9 B lymphocytes/L) in the blood and bone marrow aspirate, with atypical lymphocytes	>150 months
I	stage 0 + lymphadenopathies	101 months
II	stage 0 + splenomegaly and/or hepatomegaly associated with or without lymphadenopathies	71 months
III	anemia, with serum hemoglobin level <11 g/dL	19 months
IV	thrombocytopenia, platelet count <100,000/mm^3	19 months

TABLE 2. The BINET staging system (lymphoid areas include the laterocervical, axillary, inguinal lymph nodes, liver, and spleen)

Stage	Characteristics
A	lymphocytosis (>5 × 10^9 B lymphocytes/L) and 2 lymphoid areas affected
B	lymphocytosis (>5 × 10^9 B lymphocytes/L) and 3 or more lymphoid area affected
C	anemia (Hb <10 g/dL) and/or thrombocytopenia (Plt <100,000/mm^3) independent of the number of lymphoid area affected

TABLE 3. Chronic Lymphocytic Leukemia–International prognostic Index (CLL-IPI)

Category	Overall survival (at 5 years)	Clinical approach
Low risk	93.2 %	„Watch and wait”
Intermediate risk	79.3 %	Treat in case of symptoms
High risk	63.3 %	Treatment indication, only if the disease is active (symptomatic)
Very high risk	23.3 %	If it is possible, treat with novel agent or enroll in clinical trials

Despite affecting older age subjects, in the last years, CLL was found more frequently in younger individuals, less than 55 years of age. Based on the gender distribution, males get sick more often compared to women (male : female ratio 1.5–2 : 1).[1,2] CLL is a chronic lymphoproliferative disease, accounting for one third of adult leukemia cases and one quarter of non-Hodgkin lymphomas (NHL).[3] According to the WHO classification (World Health Organization, 2008), CLL is an indolent lymphoproliferative disorder, composed by small, mature, monomorphic, monoclonal B-cells accumulating in peripheral blood, bone marrow, and lymphoid organs.[4] The monoclonal character of these cells is based on the particular immunophenotype that includes specific cellular surface markers (CD5 – T-cell antigen; CD19, CD23, and CD20 as B-cell antigens). On the other hand, the WHO states that the difference between CLL and small lymphocytic lymphoma (SLL) is only in the leukemic appearance.[5]

DIAGNOSIS OF CLL

The positive diagnosis of CLL is based on blood smears, full blood cell count, and immunophenotyping from peripheral blood.[5]

According to the International Workshop on Chronic Lymphocytic Leukemia (IWCLL),[6,7] updated by the National Cancer Institute-Working Group (NCI-WG) guidelines,[7,8] CLL diagnosis is based on the presence of documented lymphocytosis (≥5 × 10^9 B lymphocytes/L) in the peripheral blood in the last 3 months; and secondly, flow cytometry showing a specific immunophenotypic outline: CD5 and CD23 expression with a low-level expression of CD20, CD79b, and surface immunoglobulin, as well as clonal light chain restriction (either kappa or lambda).

During the analysis of the peripheral blood smear, the leukemia cells present as healthy, mature, small lymphocytes with little cytoplasm and a dense nucleus with aggregated chromatin, without recognizable nucleoli.[5,6]

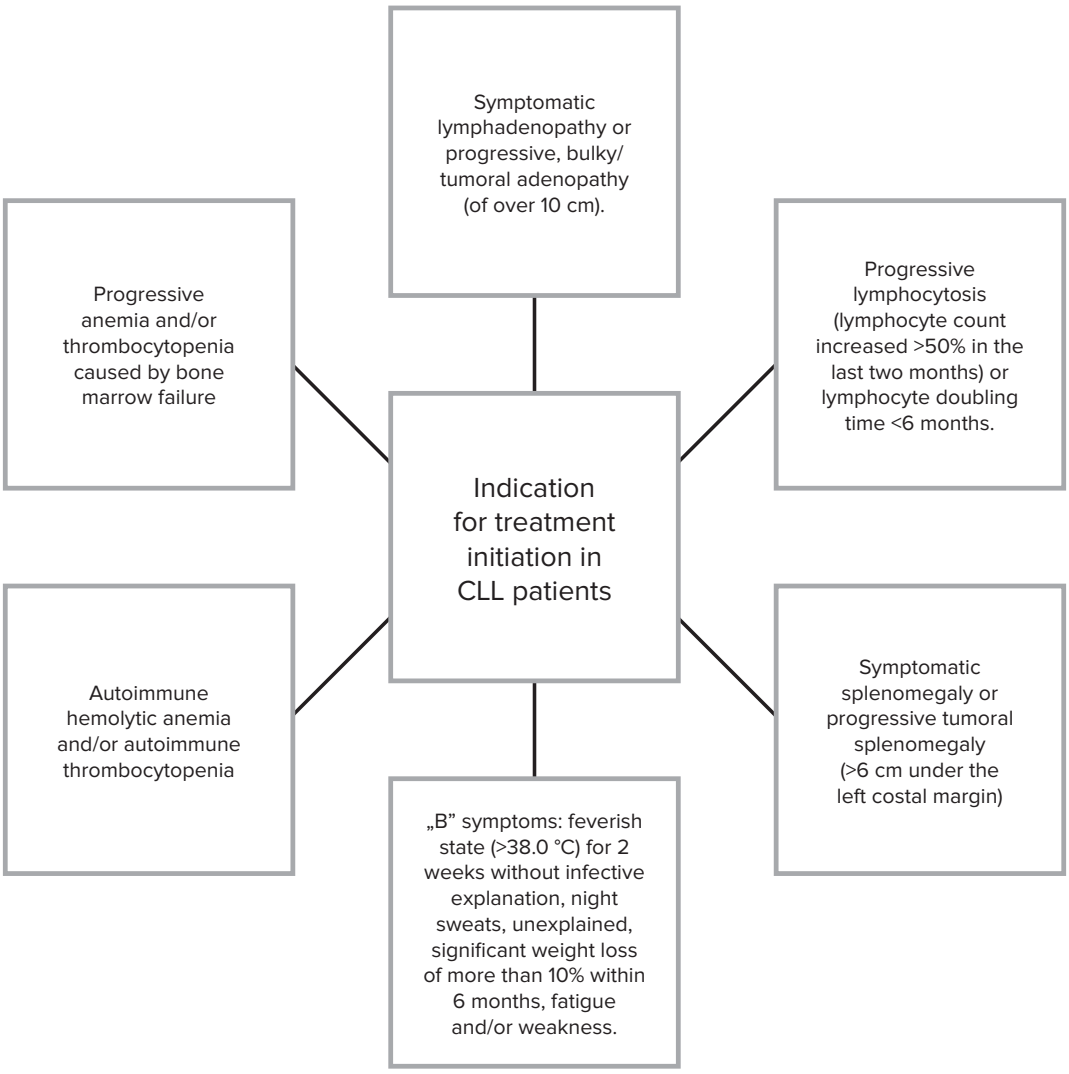

FIGURE 1. Indications for treatment in CLL according to the International Workshop on Chronic Lymphocytic Leukemia Guidelines

Hallek *et al*. and Scarfo *et al*. mention two clinical entities: small lymphocytic lymphoma and monoclonal B-cell lymphocytosis, which must be distinguished from CLL by assessing the signs and symptoms and the count of B lymphocytes in the peripheral blood. The diagnosis of lymphocytic lymphoma involves the presence of less than 5×10^9 B lymphocytes/L in the peripheral blood and is clinically characterized by lymphadenopathies and/or enlarged spleen and liver, without bone marrow infiltration-related cytopenia, requiring, in some cases, histopathologic examination from a lymph node. Monoclonal B-cell lymphocytosis is confirmed by a B-lymphocytes blood count under 5×10^9/L, in the absence of lymphadenopathies, hepatosplenomegaly, disorder-related cytopenia, or B symptoms.[5,7]

STAGING AND RISK STRATIFICATION OF CLL

In current clinical practice, the RAI and BINET staging systems are available for defining disease prognosis and indication for treatment in CLL patients.[9,10]

The RAI staging system includes 4 stages that present the median survival according to the laboratory and clinical features of the disease, and it allows classification of patients into three risk categories (Table 1). Low-risk patients present RAI stage 0, intermediate risk includes stages I and II, while high-risk patients include stages III and IV, with anemia and/or thrombocytopenia.[9]

The BINET staging system categorized patients into 3 classes, according to the B-lymphocyte count and the number of affected lymphoid regions, and the hemoglobin and platelet count (Table 2).[10]

The disadvantage of the two staging systems is that they allow the identification of only three prognostic subgroups. However, in the last two decades, genetic studies have isolated several genomic and chromosomal modifications in patients with CLL that present prognostic value irrespective of the clinical stage. In consequence, an international consortium of study groups has developed a relevant prognostic score — the Chronic Lymphocytic Leukemia-International Prognostic Index (CLL-IPI). In this staging system, five independent prognostic factor were included: age, clinical stage, serum β2-microglobulin levels, IGHV mutational status, and del(17p) and/or TP53 mutation.[5,7,11]

THERAPEUTIC GUIDELINES — INDICATION FOR TREATMENT

The two main questions to be answered before commencing a proper patient management in CLL are "When?" and "How?".[12] The International Workshop on Chronic Lymphocytic Leukemia clearly defined the signs and/or symptoms of active disease that has indication for prompt treatment initiation (Figure 1).[6,7,11]

PROPER SELECTION OF TREATMENT REGIMENS

When faced with a diagnosis of CLL, the clinician should assess the proper timing and method, either in monotherapy or with combination drug regimens, for initiating the therapeutic management of such patients. Therefore, several parameters should be evaluated, which include (1) the fitness of the patient (age, comorbidity, performance status, comorbidity index of rating scale – CIRS, renal function); (2) the stage of the disease (RAI and BINET); (3) disorder-related symptoms; (4) gene mutations [del (17p) and or TP53 mutation]; (5) previous treatment, as well as the presence of relapse or refractory disease to the last administered treatment regimens.[5,7,11]

Considering the 5 parameters previously listed, there are different possible treatment modalities.

The "Watch and wait" conduct

Delayed treatment initiation can be applied in case of CLL patients with RAI stage 0 to I and BINET stage A and B, irrespective of the gene mutations and fitness.

First-line therapy

In fit patients ("go go"), with normal organ and kidney function, with a creatinine clearance of >70 mL/minute, with a CIRS of ≤6, the current procedure is to apply standard combination chemotherapy with fludarabine, cyclophosphamide and CD20 monoclonal antibody – rituximab (FCR). Two comprehensive studies have shown an outstanding response rate with this scheme, with an overall response rate of 90% and a rate of complete response of 40%.[12,13] But the toxicity of this combination may lead to a greater incidence of grade 3 or 4 neutropenia and bacterial or viral infections within the first 2 years after treatment.[14,15] Unfortunately, there is no suitable data on methods for toxicity reduction and the only way is to reduce the treatment cycles during minimal residual disease (MRD).[16] An alternative treatment may be the combination of bendamustine + rituximab (BR).[16] At the same time, a randomized GCLLSG trial (2013) showed that the BR combination presents a better clinical tolerance, but the clinical remission rate and progression-free survival were shown to be inferior to that of the FCR combination.[18]

In unfit patients, ("slow go"), with damaged organ functions (creatinine clearance less than 70 mL/minute) and several relevant comorbidities (CIRS score >6), the therapeutic method of choice is the chlorambucil (alkylating agent) and anti-CD20 antibody combination. In 2014, Goede *et al.*, used the chlorambucil + obinutuzumab (anti-CD20 antibody) scheme and were able to prove a higher clinical response rate compared to chlorambucil monotherapy and a longer progression-free survival; however, the authors did not study the side effect profile of this regimen.[19] In 2013, Hillmen *et al.* reported similar results with the use of chlorambucil and ofatumumab combination.[20] According to Foon *et al.* (2009), the FCR-lite regimen consists in reducing the doses of fludarabine, cyclophosphamide and rituximab down to the patient's tolerability.[21]

High-risk patients, with del(17p) and/or TP53 mutations present a negative, reserved prognosis, but the recommendations are not clear about the standard therapy, which underlines the importance of patient enrollment in clinical experiments with novel drugs.

Currently, new kinase inhibitors (ibrutinib and idelalisib) are approved by the Food and Drug Administration and the European Medicines Agency as the main treatment line in patients with 17p deletion and/or TP53 mutation.[5,7]

Ibrutinib is an inhibitor of Bruton-tirosine kinase, linked irreversibly to B-cell receptor, which acts on this pathway by inducing apoptosis and blocking the proliferation process. Its adverse effects include coagulopathies (manifested with bleeding), gastrointestinal effects (e.g., diarrhea), and also CYP3A4 interactions, thus indicating careful administration in association with other inducers

of inhibitors of cytochrome p450. Also, it had also been proven useful in relapsed/refractory CLL.[22–24]

Idelalisib is a phosphoinositide 3-kinase (PI3K) inhibitor, with oral administration, and as side effects it causes hepatic cytolysis, lung involvement, and colitis with diarrhea.[25,26] This drug presents first-line indication in patients with del(17p), and, on the other hand, it can be associated with anti-CD20 monoclonal antibody (rituximab) for relapsed or refractory CLL.[27]

Second-line therapies (for relapsed and refractory CLL)

This category of patients is a gray zone too. But the recommendations are the same for patients who have received a first-option immunochemotherapy combination (such as FCR) and they relapse after more than 24 to 36 months.[12] On the other hand, if the relapse occurs within 24–36 months after the end of the treatment, or the subjects are refractory to the fludarabine-based combination (with non-progressive or progressive disease or patients who relapse within 6 months after the end of treatment), the option of clinical trial enrollment should be taken into account. In such cases, there are several possibilities of treatment. The use of alemtuzumab and fludarabine combination has been shown by Elter *et al.* (2011) to reach a response rate of more than 80% and a clinical remission in 30% of cases.[28] The anti-CD20 monoclonal antibody in association with corticosteroids were proposed by Castro *et al.* (2008), by administering rituximab in days 1, 8, and 15, with high doses of methylprednisolone (1 g/m^2) over a 4-week period.[29] Ibrutinib and idelalisib (without or with rituximab) have also been proved as indication in this patient category,[23,27] ofatumumab is a second-generation anti-CD20 monoclonal antibody that binds to a different epitope of the CD20 surface marker. It shows a tardive dissociation, which leads to a better, more efficient complement-dependent cytotoxicity. This effect is superior compared to rituximab. In double-refractory (resistant to fludarabine + alemtuzumab) CLL patients, ofatumumab is a suggested treatment possibility.[30]

Allogeneic stem cell transplantation

Sutton *et al.*, Montserrat *et al.*, and Magni *et al.* have shown that autologous stem cell transplantation dose not present a superior, significant effect on the general survival rates and on the progression-free survival, in comparison to standard immunochemotherapy schemes (e.g., fludarabine, cyclophosphamide, and rituximab regimen).[31–33] In young CLL patients with refractory disease, with or without genetic abnormalities (17p deletion and/or TP53 mutation), allogeneic stem cell transplantation should be taken into consideration.[34] This statement is encouraged by the fact that although new pharmacological agents (tyrosine-kinase inhibitors and new-generation monoclonal antibodies) have reformed the treatment modalities in CLL, there has yet to be sufficient information on their long-term efficacy and safety. As a final thought, in these patients, after the right novel agent has been chosen and has led to an optimal response, there are two options for furthering the treatment. The first option consists in performing allogeneic stem cell transplantation in order to conserve the clinical response, and the second option is to delay the cell therapy and to continue the initiated treatment with the novel drug.

OUTLOOK ON THE FUTURE OF CLL TREATMENT

Over the last two decades, there have been multiple developments that triggered a change in the therapeutic management of CLL. Due to several genetic investigations, clinicians are mapping the genetic background, which has led to a better understanding of why this indolent lymphoproliferative disease presents a slow progression in some patients (with a longer period of inactivity of the disease), while other patients should be treated shortly after diagnosis, by initiating proper therapeutic measures.

CONCLUSIONS

Comprehensive clinical studies have generated new personalized guidelines, which indicate “when” and “how” to treat patients with chronic lymphocytic leukemia. Novel pharmacological agents have significantly improved clinical outcomes in high-risk, relapsed, or refractory CLL. At the same time, there are still unanswered questions regarding the safety and long-term efficacy of such novel therapies, which require further clinical studies that may be able to provide clear answers.

CONFLICT OF INTEREST

Nothing to declare.

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ORIGINAL RESEARCH

CARDIOLOGY // HEMATOLOGY

Imaging-derived Biomarkers Associated with Atrial FIBROsis, Structural Remodeling and the Risk of Cardio-embolic Events in Patients with Atrial Fibrillation – the FIBROS Study

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ABSTRACT

Recent studies demonstrated that despite restoration of the sinus rhythm, patients with a positive history of atrial fibrillation (AF) are still at risk of thromboembolic events. The primary objective of this study is to identify new imaging-derived biomarkers provided by modern imaging technologies, such as cardiac computed tomography angiography, delayed enhancement magnetic resonance imaging, or speckle tracking echocardiography, as well as hematological biomarkers, associated with the risk of intracavitary thrombosis in patients with AF, in order to identify the imaging-derived characteristics associated with an increased risk of cardio-embolic events. Imaging data collected will be post-processed using advanced techniques of computational modeling, in order to fully characterize the degree of structural remodeling and the amount of atrial fibrosis. The primary endpoint of the study is represented by the rate of thromboembolic events. The rate of cardiovascular death, the rate of major adverse cardiovascular events, and the rate of AF recurrence will also be determined in relation to the degree of structural remodeling and atrial fibrosis.

Keywords: atrial fibrillation, atrial fibrosis, inflammation, coagulation, stroke, thrombosis, magnetic resonance, ablation

INTRODUCTION

Atrial fibrillation (AF) is the most common sustained arrhythmia that can appear in patients with or without cardiac comorbidities.[1] The incidence of AF is decreasing; however, its prevalence remained constant, one third of the adult population being affected by this devastating disease.[2,3] The risk of stroke in patients with AF decreased in the last few years; however, this was not accompanied by a decrease in mortality risk.[2] Recent studies have demonstrated that despite sinus rhythm restoration, patients with a positive history of AF are still at risk of thromboembolic events. Therefore, blood stagnation in the atria is not the only contributor to the development of intracavitary thrombosis.[4–6] In the ASSERT study, only 8% of patients with stroke or systemic embolism had atrial fibrillation in the last 30 days prior to the embolic event.[7] Along with atrial stasis, several mechanisms are involved in the pathogenesis of atrial thrombosis in AF such as atrial fibrosis, epicardial adiposity,[8,9] local inflammation, hypercoagulability,[10] endothelial dysfunction, structural pathologies, neurohumoral and genetic factors.[4] It has been described in the literature that atrial fibrosis significantly increases the incidence of stroke, and that the degree of atrial fibrosis is significantly higher in patients with stroke.[7] Figure 1 presents the main factors involved in the pathophysiology of AF and the link between AF and stroke.

Atrial fibrosis plays an important role in the appearance, maintenance, and recurrence of AF and in the effectiveness of catheter ablation. At the same time, atrial fibrosis is a consequence of AF, showing a vicious circle in which "AF begets AF".[11] Atrial fibrosis can be caused by: atrial fibrillation, rapid atrial myocyte depolarization,[12] inflammation, mechanical stretch, atrial distension,[13] cardiac injury,[11] electrical derangements,[11] accumulation of intracellular Ca ions, autocrine and paracrine mediators,[12] oxidative stress,[14] diabetes, obesity,[15] hypercoagulability (activation of PAR1 receptors by thrombin),[16] genetic factors and systemic autoimmune disease. Cardiovascular comorbidities and age (<75 years) did not appear to correlate with the degree of atrial fibrosis.[17] Atrial fibrosis can be noninvasively assessed and quantified using delayed enhancement magnetic resonance imaging (DE-MRI).[18]

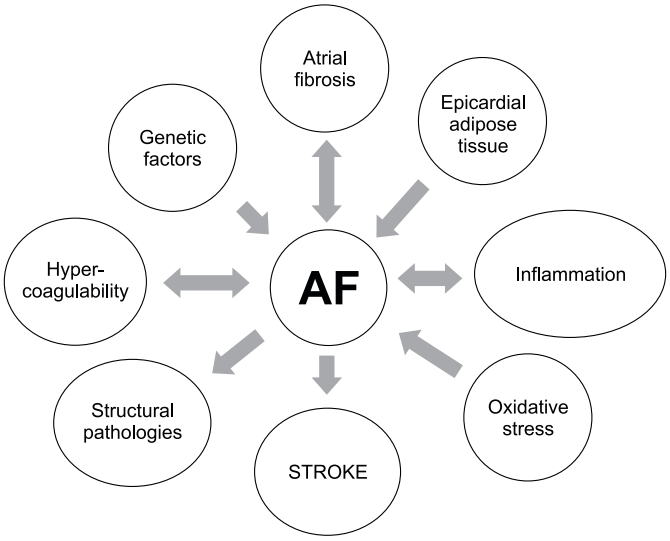

FIGURE 1. Mechanisms involved in the determinism of atrial fibrillation and associated stroke

Inflammation can be a cause or a consequence of AF. A correlation between AF and circulatory levels of inflammatory biomarkers, such as C-reactive protein (CRP), cytokines, interleukin, complement, and activation state of leukocytes and atrial fibrillation has been documented.[19,20] The link between inflammation and AF includes atrial fibrosis as a main component. Inflammation increases the level of pro-inflammation cytokines in the blood, such as IL 1, IL 2, IL 6, IL 8, CRP, tumor necrosis factor α, monocyte or chemoattractant protein 1, which stimulate endothelial and other cells.[19] An independent correlation between the level of inflammatory biomarkers and AF was described, the level of CRP and IL 6 correlating with structural remodeling (left atrium enlargement) and with impaired left atrium (LA) function.[21] Furthermore, the level of CRP is an independent predictor of AF, and this level is higher in patients with persistent AF.[19] Inflammation also has an important role in thrombus formation and ischemic events, this correlation being mediated by endothelial injury, platelet activation, tissue factors, von Willebrand factor, fibrinogen, and P-selectin.[22,23] Therefore, CRP can become a useful biomarker to assess the risk of thromboembolic events in patients with AF.[12]

Pericardial adipose tissue is a highly metabolically active tissue that can predispose to AF, involving inflammatory cytokines and oxidative stress. Furthermore, it has a quantitative association with the severity of AF.[24,25]

AF produces a hypercoagulable state. Thrombin can initiate a pro-fibrotic, pro-inflammatory, and pro-hypertrophic state via stimulation of protease-activated receptors.[26] New anticoagulant therapy can inhibit PAR1 activation and through it can prevent the development of a substrate for AF.[27]

A CHADS2 and CHA2DS2-VASC score of 0 may be insufficient to avoid thromboembolism events in patients with AF.[28] More information about thromboembolic risk can be obtain by determination of the level of troponin I, CRP, and NT-pro BNP.[29] However, even these hematological biomarkers fail to fully predict the risk of thromboem-

bolism.[28] Novel studies demonstrated that AF and atrial fibrosis are independent risk factors for stroke, even after the sinus rhythm has been restored. A degree IV (>75%) atrial fibrosis can have a huge impact on the risk of stroke and may be included in the new stroke prediction index.[13]

POTENTIAL CONTRIBUTIONS OF THE STUDY

The phenomenon of atrial fibrosis as a pathogenic mechanism of AF has been described in the literature; however, its role in atrial thrombosis has not been elucidated so far.

The originality of the present study consists in building an algorithm of investigation by which high-quality imaging data will be correlated with hematological markers of inflammation, platelet aggregation, and clotting. A model of complex investigations will be developed to identify patients with increased thromboembolic risk, and thus a large number of cardio-embolic events will be prevented.

STUDY HYPOTHESIS

The degree of atrial fibrosis and the level of inflammatory markers in the blood can predict the risk of thromboembolic events in patients with atrial fibrillation.

STUDY OBJECTIVES

Primary objective

The primary objective of this study is to identify new imaging-derived biomarkers provided by modern imaging technologies such as cardiac computed tomography angiography (CCTA), delayed enhancement MR imaging (DE-MRI), or speckle-tracking echocardiography (STE), as well as hematological biomarkers, associated with the risk of intracavitary thrombosis in patients with AF, in order to identify the characteristics associated with an increased risk of cardio-embolic events.

Secondary objectives

This study also aims to evaluate the correlation between the structural remodeling of the left and right atria and the amount of myocardial fibrosis of the left atrium, using DE-MRI. We will determine the function of the left atrium using exercise stress test (EST) and the automated quantification of atrial fibrosis using dedicated software.

At the same time, volumetric assessment of epicardial adipose tissue will be performed in each patient undergoing CCTA and MRI.

We will also look for identification of hematological biomarkers of predisposition for thrombosis and platelet aggregation and evaluate the differences between these biomarkers in blood samples collected from a peripheral line and from the left atrium during interventional ablation procedures. Another important part of this study is to analyze the electrical remodeling of the atrium using a three-dimensional electro-anatomic mapping system and to correlate these findings with the rate of thrombosis and with the level of local hematological markers. We will determine the rate of recurrence of AF, the rate of thromboembolic events, and the rate of major adverse cardiovascular events (MACE), every 3 months during the follow-up.

METHODS

Study design

This is a prospective, descriptive, cohort study composed of two major parts. In the first part of the study, laboratory tests and necessary interventions will be performed. The second part is represented by the follow-up of patients for 2 years and will contain the analysis of the data obtained during the first part of study. After evaluating eligibility for the screening process, patients who meet the inclusion criteria without exclusion criteria will be included in the study. The study population will be comprised of a minimum of 50 patients. Each patient included in the study will need to be eligible for catheter ablation. Based on the degree of atrial remodeling (size, wall thickness, and function) assessed with CCTA, the study population will be divided in 2 groups. Patients with mild atrial remodeling will be enrolled in the first group, while the second group will contain patients with moderate or severe atrial remodeling.

Personal data of patients will be collected at the start of study. Anamnesis, physical examination, ECG, evaluation of risk factors and comorbidities will be performed in each case. Lab tests will include the level of leukocytes, hs-CRP, and the erythrocyte sedimentation rate. In each case we will exclude the presence of an intracavitary thrombus using transthoracic and transesophageal echocardiography. The structure of atrial anatomy and the level of epicardial adipose tissue will be examined with echocardiography and CCTA. EST will be used for the assessment of cardiac function. Electrophysiological study will be performed in each patient included in the study and the images obtained with CCTA will be merged with the electrical map of the heart.

After trans-septal puncture, but before pulmonary veins isolation, we will harvest blood from the left atrium to determine the level of pro-inflammatory and pro-coagulation

factors. We will quantify the level of hs-CRP, IL-1,6, fibrinogen, tumor necrosis factor, the erythrocyte sedimentation rate, INR, PT, and PT%. At the same time, these factors will be determined from the peripheral blood.

The degree of atrial fibrosis will be assessed using DE-MRI. Upon discharge, we will perform a new ECG to confirm the success of cardioversion. All patients without AF at discharge will be followed-up for 2 years. Patients will be recalled for periodic investigations (anamnesis, physical examination, ECG, echocardiography) in the 3rd, 12th and 24th month and contacted by phone in the 6th and 9th month after cardioversion. At the last session (month 24), MRI and EST will be performed to assess the progression of atrial fibrosis and the changes in atrial function.

INCLUSION AND EXCLUSION CRITERIA

Patients are eligible if they had non-valvular paroxysmal or persistent AF. All patients need to be adults and be able to read and understand the informed consent document. The study cannot be carried out without imaging techniques, therefore patients who present contraindications to imaging tests will be excluded from the study. These conditions are represented by claustrophobia, hypersensitivity to contrast agents (gadolinium, CT contrast agents), pregnancy, acute or chronic kidney failure (stage 3a, 3b, 4, 5), and decompensated cirrhosis. The presence of metallic foreign bodies or cardiac rhythm device are contraindications for magnetic resonance imaging and therefore for the study.

Patients receiving any drug that may affect the level of hematological markers will be excluded from the study. Terminally ill patients and those who may not adhere or may not complete follow-up or do not have reliable information will be also excluded from the study.

ENDPOINTS

The primary endpoint of the study is represented by the rate of thromboembolic events. The rate of cardiovascular death, the rate of MACE, and the rate of AF recurrence will also be determined in relation to the degree of structural remodeling and atrial fibrosis.

DATE STORAGE AND ANALYSES

A dedicated database with the patients' data and imaging tests will be created and handled with the utmost accuracy and confidentiality, only the staff involved in the research having access to this database. Imaging data stored in the database will undergo complex post-processing in the computational medicine laboratory, using computational simulations and advanced imaging techniques processing. The merging of images obtained with CT scanner with the electro-anatomical map of the left atrium will be performed in real time during the catheter ablation procedure. The quantification of the left atrium fibrosis will be performed by a radiologist.

The statistical analysis will be performed in the medical statistics laboratory of the Center of Advanced Research in Multimodal Cardiac Imaging of SC Cardio Med SRL.

ETHICS

All study procedures are in line with the principles of the Declaration of Helsinki. All patients will sign an informed consent prior to be enrolled in the study. The study received Ethics approval from both institutional boards (approval no 28/28.12.2017 from the Ethics Committee of Cardio Med Medical Center, and approval no. 348/13.12.2017 from the Ethics Committee of the University of Medicine and Pharmacy of Tîrgu Mureș).

CONCLUSION

In AF undergoing complex ablation procedures, the rate of recurrence and the cardioversion succession rate are influenced by several intra- and extra-cardiac factors. Novel studies have shown that in addition to atrial stasis, many factors are involved in the appearance of intra-atrial thrombosis. Patients with positive history of AF have also an increased risk for stroke compared to those who never had atrial fibrillation. It is known that atrial fibrosis, inflammation, and hypercoagulability have an important role in the appearance of intracavitary thrombosis, but the exact mechanisms involved in this correlation have not been elucidated so far. This study will characterize new imaging-derived biomarkers to correlate the structural remodeling and fibrosis of the left and right atrium with the hematological parameters reflecting a high coagulability in the atria, in order identify new tools for predicting the risk of thromboembolic events in patients with AF.

ACKNOWLEDGEMENT

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funding from the University of Medicine and Pharmacy of Tîrgu Mureș, Romania, via governmental funds for PhD studies.

CONFLICT OF INTEREST

Nothing to declare.

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Journal of Interdisciplinary Medicine

ORIGINAL RESEARCH

HEMATOLOGY // INTERNAL MEDICINE

Post Autologous Stem Cell Transplantation Complication Management in Case of Malignant Lymphoma Patients

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ABSTRACT

Introduction: Autologous hematopoietic stem cell transplantation (ASCT) represents a standard therapy in the management of hematologic malignancies such as malignant lymphomas and has used for approximately three decades. The aim of this study was to determine the occurring post-ASCT complications and their impact on the patients' recovery for a better management. **Material and methods:** An observational retrospective study was performed during a five-year period between 2012 and 2017, involving 58 classical Hodgkin lymphoma and 36 non-Hodgkin lymphoma patients, who underwent ASCT in the Bone Marrow Transplantation Unit of Tîrgu Mureş. The main analyzed complications were: infections, bleeding, hydroelectrolytic disorders, and hypoalbuminemia. **Results:** After data analysis we found that 17 patients (18%) presented microbiologically confirmed infection, 10 patients (11%) presented clinically non-significant bleeding, 39 patients (42%) presented electrolyte disorders, and 33 patients (36%) presented hypoalbuminemia, obtaining a positive correlation between the rate of adverse events after ASCT with age ($r = 0.9914$, $p = 0.0009$) and the average hospitalization period ($r = 1$, $p < 0.00001$). **Conclusions:** The identification of adverse events and their correlation with the patients' clinical outcome can lead to better patient management and a faster recovery after ASCT.

Keywords: malignant lymphoma, autologous stem cell transplantation, complication management

INTRODUCTION

Autologous hematopoietic stem cell transplantation (ASCT) represents a standard therapy in the management of hematologic malignancies such as malignant lymphomas and has been used for approximately three decades.[1,2] The standard first-line chemotherapy used in Hodgkin lymphomas consists of 12 applications of the ABVD (doxorubicin, bleomycin, vinblastin, dacarbazine) regimen. In case of non-Hodgkin lymphomas, the first line of chemotherapy includes 8 courses of the CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and pred-

nisone). In both subtypes of lymphomas, the first-line chemotherapy can be combined with targeted antibody therapy (e.g., rituximab + CHOP) and radiation treatment. Despite the new era of novel targeted drugs, ASCT still remains a significant treatment method for relapsed or chemoresistant Hodgkin and non-Hodgkin lymphomas.[2–4] Due to the high-dose conditioning regimens, post-ASCT complications, such as viral and bacterial infections, can have a major impact on clinical outcome and the patients' recovery.[5,6]

The present study aimed to determine the complications occurring after ASCT, as well as their impact on the patients' recovery for a better patient management.

MATERIAL AND METHODS

An observational retrospective study was performed during a five-year period between 2012 and 2017, involving 58 classical Hodgkin lymphoma patients and 36 subjects with non-Hodgkin lymphoma, who underwent ASCT in the Bone Marrow Transplantation Unit of Tîrgu Mureş. All the enrolled patients benefited from standard-dose CEAM conditioning regimen (CCNU, etoposide, ara-c, melphalan). The main analyzed complications were: infections, bleeding, electrolyte disorders, and hypoalbuminemia. For each enrolled subject, lingual, nasopharyngeal, and urinary culture was collected for microbiological analysis before and after ASCT. In order to determine the presence or absence of hydroelectrolytic disorders and hypoalbuminemia, daily serum biochemistry analysis was performed. The obtained data was recorded in the patients' personal files. In order to identify the statistical relation between the presence of complications and the patients' age, as well as the number of complications and the hospitalization period, the Pearson Correlation Coefficient Calculator was used. The statistical significance was set at an alpha value of 0.05.

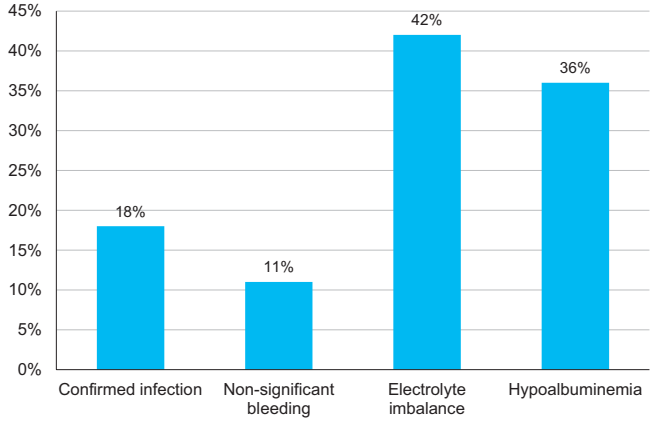

FIGURE 1. The rate of post-ASCT complications in the study population

RESULTS

After processing all data regarding our study group, we found that most patients presented electrolyte disorders, followed by hypoalbuminemia, microbiologically confirmed infections, and clinically non-significant bleedings (Figure 1).

According to the number of complications present in each patient, the study lot was divided in five groups: patients without complications, and subjects with one, two, three, or four simultaneous complications. Regarding to the above-mentioned groups, the following distribution was found: 23 patients did not present any complication (24.4%), 28 patients with one adverse event post-ASCT (29.7%), 20 patients with two complications (21.3%), 14 out of 94 patients (14.9%) presented 3 complications, while 9 patients (9.6%) from the study lot presented all four of the examined adverse events (Figure 1). The correlation coefficient between the number of complications and the median age for each group was $r = 0.9914$ ($p = 0.0009$), showing that age was directly proportional to the number of complications, and the results also showed a positive correlation between the complication rate and the average days of hospitalization after ASCT ($r = 1$, $p < 0.00001$) (Table 1).

DISCUSSIONS

The results of the present study found that the most frequent complication occurring after autologous hematopoietic stem cell therapy in lymphoma patients was the presence of electrolyte disturbances, followed by low serum albumin levels and bacteriologically confirmed infections. The rarest complication found in our study cohort was the presence of non-significant bleedings. In case of bacteriological evidence of infection, targeted antibiotic therapy was initiated. In case of bleeding, supportive plate-

TABLE 1. The median age and post-ASCT hospitalization period of each study group according to the number of complications

Number of complications	None	1	2	3	4
Median age (years)	31.6	34.1	37	41.2	45.8
Hospitalization period (average number of days)	14.4	15.2	16.1	16.9	17.7

let and fresh frozen plasma transfusion was administered, over and above in persisting active bleeding recombinant human coagulation factor VIIa (NovoSeven®) was dosed. Substitutive therapy was initiated in case of hydroelectrolytic disorders or hypoalbuminemia.

A study conducted by Jones *et al*. in the Anderson Cancer Center in Texas, focusing on post-ASCT complications during hospitalization and their impact on hospitalization costs, had highlighted the importance of this question. The results found a strong correlation between the presence of adverse events and increased costs.[7] A recently published research by Otrock *et al*. examined adverse events related to cryopreserved stem cell infusion and demonstrated that most of these immediate adverse events can be attributed to dimethyl-sulfoxide (DMSO), but the study did not evaluate the correlation between the DMSO-induced adverse events and the patients' age or hospitalization period.[8]

CONCLUSIONS

Based on the clinical and statistical analysis, we can conclude that the presence of complications and the number of these complications show a statistically significant positive correlation with the hospitalization period after autologous stem cell transplantation. Moreover, older patients were more likely to present multiple adverse events after transplantation. The identification of risk groups among transplanted patients and a better prediction of potential complications can lead to a more efficient patient management, conducting to an improved post-ASCT clinical outcome and a cost-efficient hospitalization.

CONFLICT OF INTEREST

Nothing to declare.

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ORIGINAL RESEARCH

HEMATOLOGY // INTERNAL MEDICINE

Stem Cell Mobilization and Harvesting Failure in Case of Heavily Pretreated Patients

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ABSTRACT

Background: High-dose chemotherapy and autologous stem cell transplantation have become a standard curative treatment in various hematologic malignancies. Many factors can affect the success of mobilization and hematopoietic stem cell harvesting. **Aim:** The aim of this study was to analyze factors that lead to mobilization failure. **Material and Methods:** We conducted a retrospective study on 19 patients with failure of stem cell harvesting. All patients were administered high doses of GCS-F (filgrastim, 15 µg/kg/day) and 0.24 mg/kg of plerixafor on day +5 or +10 of harvesting. **Results:** The median age of the study population was 51 years (range 35–67) and 52.6% (n = 10) were males. The study group included 4 (21%) subjects with multiple myeloma, 6 (31.5%) with Hodgkin lymphoma, 8 cases (42.1%) with non-Hodgkin lymphoma and 1 patient with chronic lymphocytic leukemia. Each patient received 2.78 (range 1–5) lines of chemotherapy, administered in 11.57 (range 2 to over 20) cycles of treatment. **Conclusion:** In hematologic malignancies it is very important to collect stem cells in time, in order to reduce mobilization failure. As we have shown in our studied cases, multiple lines of polychemotherapy with or without radiotherapy lead to mobilization failure.

Keywords: mobilization failure, heavily treated patients, influencing factors

INTRODUCTION

Autologous hematopoietic stem cell treatment is a curative method for subjects suffering from several hematologic malignancies. In these cases, peripheral blood is the preferred source to harvest CD34+ cells.[1] The use of granulocyte-colony stimulating factor (G-CSF) alone or in combination with chemotherapy results in a collection of an adequate number of peripheral blood stem cells (PBSC). The recommended minimally sufficient number of cells is 2×10^6 CD34+ cells/kg, otherwise the procedure is associated with slower blood count recovery, a higher number of transfusion requirements, infections, and longer period of hospitalization.[2–4]

Mobilization failure is the main cause for not being able to perform autologous hematopoietic stem cell (HSC) transplantation.[5] The main factors that influence stem cell mobilization include age, previous therapy, collateral diseases, and genetic polymorphism.[5,6]

An important risk factor for hematopoietic stem cell exhaustion and mobilization failure is represented by successive cycles of chemotherapy. HSCs present changes in their quality throughout development and life, and the greatest proliferation and differentiation potential is present in HSCs obtained from the fetal liver, followed by neonatal and postnatal bone marrow of young and older donors.[7]

AIM OF THE STUDY

The objective of this study was to analyze the factors associated with mobilization failure for autologous PBSC in a group of 19 patients from a single hematology unit.

MATERIAL AND METHODS

We performed a retrospective study in the Bone Marrow Transplantation Unit of Tîrgu Mureș during a 4 year interval from January 1, 2014 to December 31, 2017. During this 4-year period, 212 patients with different hematologic malignancies and 12 donors for allogeneic transplantation underwent stem cell mobilization. From the 224 cases, 19 cases presented failure of mobilization. The present study analyzed the 19 patients with failure of SC harvesting, diagnosed with multiple myeloma, Hodgkin lymphoma, non-Hodgkin lymphoma, and chronic lymphocytic leukemia, in whom a combined mobilization method was used (GCS-F + plerixafor, chemotherapy followed by GCS-F + plerixafor). All subjects were administered high doses of GCS-F (filgrastim, 15 μg/kg/day) and 0.24 mg/kg of plerixafor on day +5 or +10 of mobilization. In all cases, the harvesting was unsuccessful, the PBSC number being under 0.5×10^6 CD34+ cells/kg body weight.

A number of 5 patients were excluded from the study because the mobilization failure was caused by other associated diseases (thrombosis, acute myocardial infarction, respiratory insufficiency, active hepatitis).

RESULTS

The study lot had a mean age of 51 years (ranging between 35–67 years) and included 52.6% (n = 10) males. The study group included 4 (21%) patients with multiple myeloma (one with IgA with previous Hodgkin disease and 3 cases with IgG secretory myeloma), 6 (31.5%) patients with Hodgkin lymphoma (3 with nodular sclerosis, 3 with mixed cellularity lymphoma), 8 cases (42.1%) with non-Hodgkin lymphoma (2 with marginal zone lymphoma, 3 with T-cell lymphoma, 2 with mantle cell lymphoma, and 1 patient with Burkitt lymphoma), and one patient with chronic lymphocytic leukemia.

The mobilization of stem cells was performed with a combination of chemotherapy + GCS-F + plerixafor in 10 cases and GCS-F + plerixafor in 9 cases.

The age distribution is presented in Figure 1, where the highest number of patients had ages between 51 and 60 years.

The mean number of chemotherapeutic lines administered to each patient in the study population was 2.78, with a range between 1 and 5.

The mean number of chemotherapeutic cycles/applications was 11.57, with a range between 2 to over 20 cycles (Figure 2).

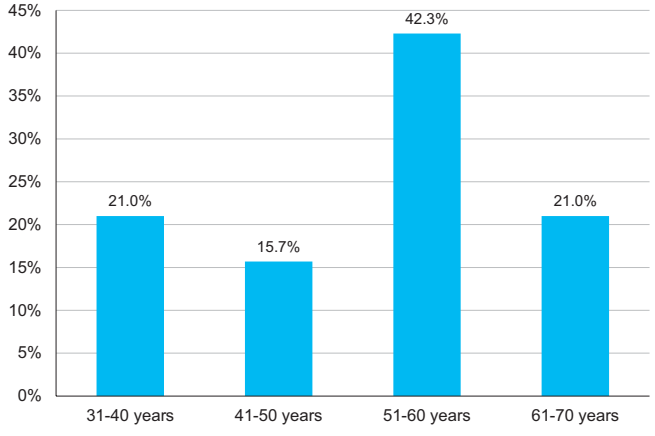

FIGURE 1. Age distribution of the study population

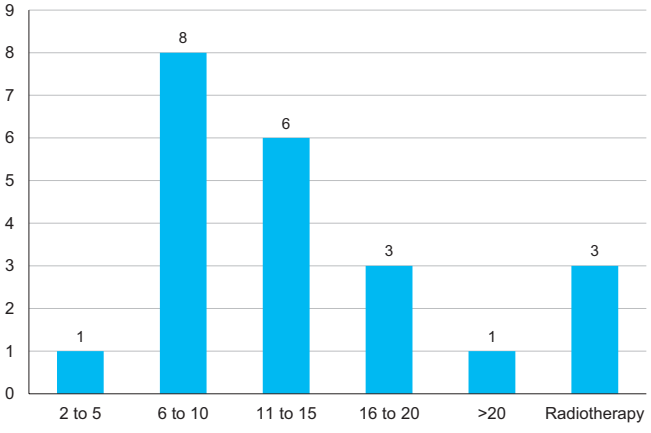

FIGURE 2. The number of chemotherapy applications in the study population

TABLE 1. Hematological parameters in patients with mobilization failure

Parameters	Range of values	Median
WBC, cells/μL	2,980–30,000	9,001
Hemoglobin, g/dL	4.8–14.6	10.83
HTC, %	17.3–42.7	32.85
Platelets, cells/μL	89,000 –723,000	227,000

WBC – white blood cell count; HTC – hematocrit

The median blood cell count prior to stem cell mobilization is illustrated in Table 1.

DISCUSSION

Our study shows that stem cell mobilization failure for autologous HSCT is highly correlated with the type of previous chemotherapy, therapy lines, and diagnosis.

All patients in the study group were heavily pretreated with alkylating agents and purine analogs. Alkylating agents were used in intermittent courses, with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP-like regimen). Other studies have found similar results, indicating that mobilization failure occurs more often when using these agents.[5,8,9]

The highest ratio of mobilization failure occurred in non-Hodgkin lymphoma (8 cases), followed by Hodgkin lymphoma (6 cases) and multiple myeloma (4 cases), similar to the results of a study conducted by Sancho *et al.*[5]

Patients with ages between 51–61 years had the highest ratio of failure.

Previous malignant disease can be mentioned as another important factor causing failure of stem cell mobilization.

A known risk factor in poor mobilization is previous radiotherapy,[5,9] which was present in only 3 cases.

By analyzing the hematological parameters, our results showed that patients with mobilization failure had moderate anemia with serum levels of hemoglobin of 10.83 g/dL and hematocrit of 32.85%, without leukopenia, neutropenia, thrombocytopenia, and serum iron overload. Other studies describe that mobilization failure is more common in patients with thrombocytopenia, neutropenia, anemia, leukopenia.[5,9,10]

CONCLUSION

Many factors can lead to mobilization and stem cell harvesting failure, making it impossible to perform autologous transplantation. We can conclude that in hematologic malignancies it is very important to collect stem cells in time, in order to reduce mobilization failure. As we have shown in our studied cases, multiple lines of polychemotherapy with or without radiotherapy lead to mobilization failure. The use of combined growth factor mobilization by adding plerixafor can increase the number of CD34+ cells, making mobilization possible in selected cases.

CONFLICT OF INTEREST

Nothing to declare.

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CASE REPORT

HEMATOLOGY // INTERNAL MEDICINE

Prognosis of Patients with Acute Myeloid Leukemia Regarding the Presence FLT3 Gene Mutation – a Case Report

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ABSTRACT

Acute myeloid leukemia (AML) is a cancerous disease affecting the myeloid line of the bone marrow cells. FLT3, also known as CD135, is a proto-oncogene, which, if mutated, leads to different types of cancer. The protein it encodes presents tyrosine-kinase activity, and its intra-tandem mutation, FLT3-ITD, leads to uncontrolled proliferation of myeloblasts and worse outcomes in AML patients. There are currently several pharmacological agents that can inhibit the effect of either the proteins with tyrosine-kinase activity or the mutated FLT3 gene. We present the case of a 68-year-old patient, smoker, with a history of arterial hypertension, chronic obstructive pulmonary disease, presenting with headache unresponsive to antalgics, dyspnea after physical exertion, and epistaxis, with onset 2 months prior to his presentation. The patient was diagnosed with AML with positive FTL3 mutation for which conventional induction therapy was initiated. Within the next days, the patient presented several complications related to the disease itself or caused by the treatment, which eventually led to his death.

Keywords: acute myeloid leukemia, FTL3 mutation, stem cell transplantation

INTRODUCTION

Acute myeloid leukemia (AML) is a cancerous disease affecting the myeloid line of bone marrow cells. It occasionally presents as tumor masses in other tissues, such as the skin and lymph nodes, when it is called myeloid sarcoma. According to the FAB classification, there are several types of AML, more specifically: M0 – with minimal differentiation, M1 – without maturation, M2 – with maturation, M3 – acute promyelocytic leukemia, M4 – acute myelomonocytic leukemia, M5 – acute monoblastic leukemia, M6 – acute erythroleukemia, and M7 – acute megakaryoblastic leukemia.[1,2]

FLT3, also known as CD135, is a proto-oncogene, which, if mutated, leads to different types of cancer. This protein presents tyrosine-kinase activity, and its

intra-tandem mutation, FLT3-ITD, leads to uncontrolled proliferation of myeloblasts.[3,4]

The treatment of AML consists of chemotherapy, supportive treatment, and hematopoietic stem cell transplantation. Chemotherapy consists of two phases: remission induction and consolidation therapy. For remission induction, idarubicin 12 mg/m^2 for 3 days and cytarabine 100 mg/m^2 is administered in combination, for a period of 7 days. In general, 2 cycles of this combination therapy are administered for a period of 3 to 4 weeks. The consolidation therapy consists in administrating 2–3 cycles of high-dose cytarabine 2000–3000 mg/m^2 for 3 days. There are also remission induction therapies with other combinations, such as mitoxantrone 7 mg/m^2 in days 1, 3, and 5, with association of cytarabine 100 mg/m^2 for 7 days. The FLAG-Ida combination therapy consists in the administration of fludarabine 30 mg/m^2 for 5 days, with cytarabine 2000 mg/m^2 for 5 days, idarubicin 10 mg/m^2 for 3 days, and the administration of granulocyte-colony stimulating factor until the normalization of the neutrophil count.[5–7]

There are currently several pharmacological agents that can inhibit the effect of either the proteins with tyrosine-kinase activity or the mutated FLT3 gene.[8] Sorafenib is an oral medication that inhibits the effect of proteins with tyrosine-kinase activity, and it was initially developed for treating hepatocellular carcinoma or renal cell carcinoma. An additional effect of sorafenib is to inhibit the mutated FLT3 gene, thus having additional benefits in the treatment of AML.[9] Quizartinib is a selective inhibitor of the FLT3-ITD mutation, and because of this, it represents an important substance in the treatment of AML with positive FLT3-ITD mutation.[10] Midostaurin, an inhibitor of proteins that hold tyrosine-kinase activity, is also an inhibitor of FLT3-ITD, but its selectivity is much lower than that of quizartinib.[11]

CASE PRESENTATION

We present the case of a 68-year-old patient, smoker, with a history of arterial hypertension, chronic obstructive pulmonary disease, presenting with headache unresponsive to antalgics, dyspnea after physical exertion, and epistaxis, with onset 2 months prior to his presentation. The routine laboratory analysis showed high leucocyte count, anemia, and thrombocytopenia. The complete clinical examination revealed the presence of emphysematous thorax, crepitus rales at the lung bases, cardiomegaly, tachycardia (heart rate of 106/minute), and high blood pressure of 150/100 mmHg.

The patient and the institution where the patient was admitted agreed to the publication of his data.

The patient underwent a bone marrow aspiration from the sternum for bone marrow analysis, which revealed the presence of 90% myeloblasts. The peripheral blood showed the presence of promyelocytes, anemia, thrombocytopenia, and absence of any kind of mature leucocyte. FLT3-ITD mutation and also MPL-RARa mutation were determined and were found to be positive. Patient management consisted in the initiation of remission induction therapy with idarubicin for 3 days and cytarabine for 7 days in adjusted doses, in association with all-trans retinoic acid 40 mg per day, motivated by the presence of promyelocytes in the peripheral blood.

After 4 days of treatment, the patient presented lower leukocyte count, but also dyspnea, headache, and pain at the level of the sternum, associated with hemoptysis. These signs and symptoms arose the suspicion of disseminated intravascular coagulation (DIC), for which the D-dimer levels were evaluated and found at a level of 27.5 mg/dL (normal range <0.5 mg/L). Because of this, low-molecular-weight heparin was initiated, in association with transfusion and fresh frozen plasma as supportive therapy. Because of febrile neutropenia, antibiotic treatment was initiated with meropenem 3 × 1 g/day, teicoplanin 2 × 400 mg/day, and colistin 4 × 2 MIU/day, as well as antiviral therapy with acyclovir 1,600 mg/day and antifungal treatment with voriconazole 2 × 200 mg/day. After 7 days of treatment, the patient presented severe headaches resistant to analgesics, followed by motor impairment of the right side of his body, which raised the suspicion of a hemorrhagic stroke. The patient was transferred to the intensive care unit, where the hemorrhagic stroke was confirmed by cranial CT examination.

The supportive therapy was continued in the intensive care unit; however, after one week, the patient had deceased.

DISCUSSIONS

The presented case is part from a larger cohort of patients with AML that were admitted in the Hematology and Marrow Transplantation Clinic of Tîrgu Mureș between 2010 and 2017, 210 subjects more specifically, out of which 10 were found to be positive for FTL3-ITD mutation. From the total number of AML patients with positive FLT3-ITD mutation, none had survived, while from the total of 210 AML subjects, the survival rate is 7 % (n = 15).

A study conducted in Toronto, Canada, published in 2012, analyzed 97 new cases of AML over an 8-year period, out of which 70 presented relapse, and 57 were tested for FLT3-ITD mutation. All patients had received reinduc-

tion therapy, 17 had undergone allogeneic bone marrow transplant, and 6 patients were positive for FLT3-ITD mutation. In total, 50 patients from the total of 70 relapse patients had deceased during the 9-month follow-up after relapse.[12]

In 2016, a case series on 4 patients with AML with FLT3 mutation was published, in which the patients were treated with chemotherapy, quizartinib, sorafenib, and allogeneic stem cell transplantation. One of the cases, a 48-year-old female patient, entered the remission phase after 4 cycles of chemotherapy, but presented relapse after 6 months for which she was treated with quizartinib and allogeneic stem cell infusion from a compatible donor. Despite having presented graft versus host disease involving the skin and liver for which she received prednisone and tacrolimus, and after a 90-day administration of sorafenib 200 mg daily, the patient was still alive at the 5-year follow-up. The authors revealed that performing allogeneic stem cell transplantation as soon as possible after achieving remission leads to a significant improvement in overall survival and longer periods of remission.[13] The patient reported in the present article was not treated with allogeneic stem cells, despite having a positive FLT3-ITD mutation, which could have been beneficial in this case.

A study performed by using data from the Center for International Blood and Marrow Transplant Research on 511 patients with de novo AML who had undergone hematopoietic stem cell transplantation found that 31% (n = 158) of subjects were positive for FTL3 mutation, which led to a significantly higher risk of relapse compared to the wild-types (38% vs. 28%, p = 0.04, RR = 1.6, 95% CI 1.15–2.22, p = 0.0048). However, the study found that the presence of FTL3 mutation was not associated with higher non-relapse mortality or overall survival, and 50% of subjects with this mutation who underwent stem cell therapy survived during the long-term follow-up.[14]

Another research on 481 patients with AML evaluated the impact of FTL3 mutations in three cytogenetic subgroups of patients: with core binding factor AML, with normal karyotype AML, and poor risk AML, respectively. The results showed no significant impact of the mutation in the first two groups, but in normal karyotype subjects FTL3-ITD mutations were associated with poorer outcomes, which were increasingly worse as the mutation burden was higher.[15]

In the above case report, the patient had been diagnosed with AML and was found to be positive for FTL3 mutation. Shortly after receiving conventional induction therapy with idarubicin and cytarabine, he presented disseminated intravascular coagulation, followed by a hemorrhagic stroke, which eventually led to death. The relatively quick worsening of the patient's status did not give time for initiation of protocols for stem cell transplantation, which could have been a therapeutic alternative in this case.

CONCLUSIONS

Positive FLT3-ITD mutation leads to a poor prognosis of patients with acute myeloid leukemia, by leading either to increased mortality and complication rates, or to a high relapse rate. Allogeneic stem cell transplantation is a viable option for AML patients with or without FTL3 mutation, leading to better outcomes.

CONFLICT OF INTEREST

Nothing to declare.

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CASE REPORT

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The Evolution of Intracardiac Hemodynamics Post Autologous Stem Cell Transplant in a Case of Multiple Myeloma Associated with Severe Tricuspid and Mitral Valve Insufficiency

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ABSTRACT

Stem cells are undifferentiated cells that can divide and become differentiated. Hematopoietic stem cells cannot transform into new stem cells such as cardiomyocytes or new heart valves, but they act through paracrine effects, by secreting cytokines and growth factors that lead to an increase in contractility and overall improved function. In this case report, we present how autologous stem cell transplantation can bring two major benefits: the first refers to hematological malignancy and the second is about the improvement of the heart condition. We present the case of a 60-year-old patient diagnosed with multiple myeloma suffering from a bi-valve severe condition in which autologous stem cell transplantation led to the remission of the patient's malignant disease and also improved the heart function.

Keywords: multiple myeloma, autologous stem cell transplantation, valve insufficiency

INTRODUCTION

Multiple myeloma was first described by Solly in 1844 and is one of the most common malignant diseases that usually appear at old age (>65 years).[1] In recent years, the incidence started to grow in younger patients. It represents approximately 10% of the hematological malignancies and 1% of all malignancies.[2] It is characterized by the proliferation of malignant plasma cells and overproduction of a monoclonal paraprotein (M protein). Until the present day, the disease is considered to be treatable, but incurable.[3] Since 1975, the Durie-Salmon staging system has been used to stratify patients with multi-

ple myeloma. Serum beta 2-microglobulin, serum albumin, thrombocyte count, serum creatinine, and age have emerged as powerful predictors of survival and have led to the creation of this International Staging System (ISS) that comprises three stages. The first stage is defined by a serum beta 2-microglobulin value of under 3.5 mg/L and albumin levels of 3.5 g/dL, in which the mean survival rate is up to 62 months; the second stage is intermediary between the first and last; and the third stage includes a level of beta 2-microglobulin of over 5.5 mg/L, leading to a median survival of 29 months.[4,5] The ISS staging method was later confirmed on subjects ≤65 years of age, in subjects with standard therapy, or autotransplantation in comparison with the Salmon/Durie staging method.[6] The treatment of multiple myeloma is very vast and includes chemotherapy, immune therapy, radiation therapy, surgery, or a combination of these, as well as the novel use of stem cell transplantation.[7–9]

Tricuspid insufficiency, also known as tricuspid regurgitation or tricuspid valve incompetence, is a valve disease described by the incomplete closure of the cusps during the cardiac systole, causing the blood to leak backwards from the right ventricle to the right atrium. It is considered to be functional (secondary) in the majority of the cases or organic (primary) in the rest. Mitral valve insufficiency or mitral valve regurgitation is a condition in which the heart mitral valve does not close tightly during the systole which causes the blood to leak upwards from the left ventricle to the left atrium.[10,11]

CASE REPORT

We present the case of a 60-year-old patient diagnosed with stage I/B λ light chain multiple myeloma, also suffering of secondary severe anemia, moderate thrombocytopenia, stage II chronic kidney disease, B-virus chronic hepatitis, and moderate/severe mitral valve insufficiency associated with severe tricuspid insufficiency, who benefited from autologous stem cell transplantation.

After the admittance, the patient presented symptoms related to congestive heart failure such as severe fatigue, dyspnea, orthopnea, jugular pulsation, hepatomegaly of stasis, and edema. The clinical examination of the cardiovascular system revealed holosystolic murmur at the apex, radiating to the axilla. The blood analyses revealed anemia, thrombocytopenia, and high levels of creatinine and urea. The ultrasound examination of the abdomen revealed stasis of the liver.

Because of the cardiotoxicity of the conditioning regimen (HD-melphalan) and because the patient was already treated with cardiotoxic drugs (cyclophosphamide), the patient was overseen by a cardiologist before administering the conditioning regimen.

The transthoracic echocardiographic examination conducted five days before the stem cell transplantation revealed the following: right ventricle diameter 36 mm, left ventricle 56/38 mm, interventricular septum 8/11 mm, posterior wall 10/13 mm, aortic annulus 19 mm, ascendant aorta 29 mm, ejection fraction 50%, left atrium area 38 cm^2, left ventricle/left atrium gradient 45 mmHg, pulmonary artery pressure 65 mmHg; mitral valve – moderate/severe regurgitation; tricuspid valve – severe regurgitation; mitral valve prolapse with moderate to severe regurgitation; aortic valve – supple, mobile, opened in M mode cusps, aortic valve opening 22 mm; pericardium with posterior echo-free space of 6 mm, with increased echogenicity; inferior vena cava 31 mm, without inspiratory collapse present and dilated suprahepatic veins. The final diagnosis of the transthoracic echocardiography included the presence of moderate to severe mitral regurgitation, severe tricuspid regurgitation, and severe pulmonary hypertension.

The standard conditioning regimen consists of HD-melphalan with a mean of 200 mg/m^2, but because of the cardiac condition of the patient the dose was lowered to 140 mg/m^2, consisting in a total dose of 274 mg melphalan. Two days after chemotherapy, the patient benefited from the reinfusion of the stem cells through a central venous catheter. The total quantity of the harvested cells was 5.54×10^6/kg body weight, and the patient benefited of 3.17×10^6/kg body weight, which is more than enough for a successful procedure. As a complication of the stem cell transplantation, the patient presented nausea, vomiting, and diarrhea, which led to electrolyte disorders, including hypokalemia and hypocalcemia, which needed rebalancing.

Twelve days after the stem cell transplantation, the patient was overseen again by the same cardiologist, and the transthoracic echocardiographic examination was repeated, with the following findings: left atrium area 32 cm^2, ejection fraction 60%, moderate mitral regurgitation, regurgitation area 6 cm^2, mild tricuspid regurgitation, pulmonary artery pressure 55 mmHg, without vegetations. This time, the echocardiographic examination concluded in a diagnosis of moderate mitral regurgitation, mild tricuspid regurgitation and mild pulmonary hypertension, and minimum pericardial liquid, thus showing significant improvement since the last echocardiography that was performed before stem cell transplantation.

The patient was released 7 days later in a good general state, with laboratory blood tests within normal range and with an improved heart condition. The patient agreed to the publication of his data and the institution where the patient had been admitted, approved the publication of the case.

CONCLUSION

Due to the pluripotent properties of stem cells, in the case presented in this article it is shown that not only the hematological malignancy represented by the multiple myeloma was efficiently treated but the heart condition was also obviously and objectively improved.

CONFLICT OF INTEREST

Nothing to declare.

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2. Nichols WW, Rourke MF. Aging, High Blood Pressure and Disease in Human. 3rd ed. London/Melbourne: Lea and Febiger; 1990.

Reference to a chapter in a book

3. Nichols WW, O'Rourke MF. Aging, high blood pressure and disease in humans. In: Arnold E, ed. McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles. 3rd ed. London/Melbourne/Auckland: Lea and Febiger, 1990; p. 398-420.

Reference to a webpage

4. Panteghini M. Recommendations on use of biochemical markers in acute coronary syndrome: IFCC proposals. eJIFCC 14. http://www.ifcc.org/ejifcc/vol14no2/1402062003014n.htm (28 May 2004)

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