

LONG-TERM EVOLUTION OF BOSENTAN TREATMENT IN SYSTEMIC SCLEROSIS PATIENTS – THE EXPERIENCE OF A PRESCRIBING CENTRE FROM BUCHAREST, ROMANIA

ANA MARIA GHEORGHIU ^{1*}, IULIANA CONEA ¹, CRISTINA VRANCIANU ¹, MARIANA SASU ¹, MIHAELA MILICESCU ¹, CARINA MIHAI ^{1,2}, MIHAI BOJINCA ¹, IOAN ANCUTA ¹

¹Internal Medicine and Rheumatology Department, “Cantacuzino” Hospital, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

²Department of Rheumatology, University Hospital Zurich, University of Zurich, Switzerland

*corresponding author: ana.gherghe@gmail.com

Manuscript received: November 2021

Abstract

Peripheral vasculopathy is a severe complication of systemic sclerosis (SSc) through digital ulcerations (DUs) that can lead to gangrene or amputations. Bosentan, an antagonist of endothelin receptor 1 with proven efficacy in preventing new onset of DUs in patients with SSc, was first introduced in Romania in 2014. Our objective was to evaluate the efficacy and long-term safety of bosentan in SSc patients in a prescribing centre in Bucharest. We included 49 patients with SSc (39 women, with a median (IQR) duration of follow-up of 25 (43) months), evaluated in our clinic between November 2014 and March 2021, who presented with DUs on admission or in the preceding three months. We compared clinical and laboratory data, including the number of DUs, Visual Analogue Scale (VAS) for Raynaud’s phenomenon and DUs, Health Assessment Questionnaire disease index (HAQ) at baseline and follow-up. At the initiation of treatment, patients presented a median (IQR) of 4 (4) DUs, VAS Raynaud 8 (2), VAS DUs 9 (3), HAQ 1.75 (1), with a significant reduction at 12 months of the number of DUs to DUs 0 (0), VAS Raynaud 2 (2.9), VAS DUs 0.5 (1.75) and HAQ 0.88 (1.13), with a maintained efficacy for DUs, VAS Raynaud and VAS DUs for the entire follow-up period. There were 8 (16.3%) cases of hepatic cytolysis 3 - 6 times the upper normal limit which required discontinuation of treatment, but otherwise no severe reactions. The treatment with bosentan was efficient on the long-term and well-tolerated by most patients.

Rezumat

Afectarea vasculară periferică este o complicație severă a sclerodermiei sistemice (ScS), prin ulcerările digitale (UD) care pot duce la gangrenă sau amputații. Bosentanul, antagonist al receptorilor endotelinei, cu eficiență dovedită în prevenția apariției UD la pacienții cu ScS, a fost introdus în România în 2014. Obiectivul studiului a fost de a evalua eficacitatea și siguranța pe termen lung a tratamentului cu bosentan la pacienții cu ScS dintr-un centru prescriptor din București. Au fost incluși în studiu 49 de pacienți cu ScS (39 femei, cu durată mediană (IQR) de urmărire 25 (43) luni în perioada noiembrie 2014 – martie 2021, care au avut UD la prezentare sau în ultimele 3 luni anterior. S-au comparat datele clinice și de laborator (inclusiv numărul de UD, *Visual Analogue Scale* (VAS) pentru fenomenul Raynaud și UD, *Health Assessment Questionnaire index* (HAQ) de la inițierea terapiei până la 6 ani de urmărire. La inițiere, pacienții au prezentat 4 (4) UD, VAS Raynaud 8 (2), VAS UD 9 (3), HAQ 1,75 (1,0), cu scăderea semnificativă la 12 luni a numărului de UD la UD 0 (0), VAS Raynaud 2 (2,9), VAS UD 0,5 (1,75) și HAQ 0,88 (1,13), $p < 0,001$ pentru toate, cu menținerea eficacității pentru UD, VAS Raynaud și VAS UD pentru întreaga perioadă de urmărire. S-au înregistrat 8 (16,3%) sindroame de citoliză hepatică 3 - 6 x LSN, care au necesitat oprirea tratamentului, în rest nu a fost înregistrată nicio reacție adversă severă. Tratamentul cu bosentan a avut eficiență îndelungată și toleranță bună la majoritatea pacienților.

Keywords: systemic sclerosis, bosentan, vasculopathy, digital ulcers

Introduction

Systemic sclerosis (SSc) is a complex autoimmune connective tissue disease (CTD) characterized by immune dysregulation, early microvasculopathy and progressive tissue and inner organ fibrosis [6]. Peripheral vasculopathy is a frequently encountered complication of SSc, with clinical manifestations ranging from Raynaud’s phenomenon to critical digital ischemia and ischemic digital ulcers (DUs) [10]. It is estimated that DUs occur in 35% to 60% of patients with SSc in the course of their disease [20, 25, 28, 32]

and frequently multiple DUs occur simultaneously in several fingers of one hand or in both hands [25, 28]. DUs lead to severe pain and significant impairment of hand function and have a major impact on the quality of life of patients with SSc [9, 20]. Up to 30% of patients have severe ischemic DUs complicated by gangrene, who eventually require amputation or digital sympathectomy [20, 22, 25, 28]. Moreover, the history of DUs at presentation predicted the occurrence of DUs at follow-up and was associated with cardiovascular impairment and decreased survival in a large

study on the EULAR Scleroderma Trials and Research (EUSTAR) cohort [23]. Several mechanisms have been suggested to underlie vasculopathy in SSc, including intimal hyperplasia of small to medium-sized arteries, oxidative stress-related injury [29], endothelial cell injury and apoptosis and vascular spasm [17]. It is generally considered that intrinsic endothelial dysfunction is the central event in the pathogenesis of SSc vasculopathy [14]. Following endothelial injury, circulating platelets adhere to sub-endothelial tissue and initiate fibrin deposition and intravascular thrombus formation [12]. Endothelin-1 (ET-1), a potent vasoconstrictor, is released from endothelial cells [34]. ET-1 also stimulates fibroblast proliferation and collagen formation. ET-1 is activated by angiotensin (AT), vasopressin and transforming growth factor β . In turn, AT has both vasoconstrictive and profibrotic properties [26, 33]. Increased plasma ET-1 and ET-1 receptor expression have been associated with Raynaud's phenomenon and SSc [4], suggesting a critical role in vasculopathy. The approach for treating Raynaud's phenomenon and DUs is focused on targeting these structural abnormalities by preventing the cause of vascular injury. Such treatment strategies include the use of calcium channel blockers (CCBs), phosphodiesterase (PDE) inhibitors, prostacyclin analogues and ET receptor antagonists [1, 26]. Other medicines, such as AT enzyme inhibitors and receptor blocking agents have been employed in the treatment of secondary Raynaud's phenomenon due to their deterrent effect on AT-II [1], but do not seem to have any effect in the treatment of DUs [1].

Bosentan is a dual ET receptor antagonist with established efficacy on preventing new onset of digital ulcerations recommended especially for patients with multiple, persistent DUs despite the use of CCBs, PDE-5 inhibitors or iloprost therapy [15, 19], but its long-term effect is insufficiently studied. In Romania, it is currently the only drug approved for the prevention of new SSc-related DUs in patients unresponsive to standard therapy since 2014 in 8 selected prescription centres through the National Program for Rare Diseases – prevention of DUs in patients with SSc [5].

The objectives of the study were to assess the long-term efficacy and safety of bosentan in patients with SSc-related DUs in a prescribing centre in Bucharest, Romania.

Materials and Methods

Study design

Forty-nine adult patients diagnosed with SSc, who fulfilled the 2013 American College of Rheumatology (ACR)/Eular League Against Rheumatism (EULAR) Classification Criteria for SSc [31] who were evaluated and started the therapy with bosentan for the prevention of DUs in the Department of Internal Medicine and Rheumatology of "Cantacuzino" Clinical Hospital,

Bucharest, Romania, between November 2014 and March 2021, were included. All patients had positive antinuclear antibodies (ANA) and an SSc-specific capillaroscopy pattern and presented ischemic DUs (defined clinically as an area of denudation of at least 1 mm, with a loss of continuity of epithelial coverage) either on admission or at least one recurrent DU in the three months prior to admission, which did not respond to standard first-line therapy with calcium-channel blockers at the maximal tolerated dosages. Patients were prescribed bosentan 62.5 mg orally b.i.d. (*bis in die*) for the first 4 weeks, thereafter 125 mg b.i.d., according to the national prescription protocol [5]. As *per* protocol, digital scars, history of gangrene or amputation and extrusion of subcutaneous calcifications were not considered indications for bosentan treatment. Contraindications to treatment were: hypersensitivity to bosentan or to any of the excipients, moderate to severe hepatic impairment (i.e. Child-Pugh class B or C), initial plasma concentrations of aminotransferases (AST and/or ALT) 3-fold higher than the upper limit of normal values, concomitant use of cyclosporine, pregnancy or not using safe contraception in women of childbearing potential. The primary endpoint of treatment was at least 50% reduction in the number of new DUs at 24 weeks; secondary endpoints were at least 50% decrease in Visual Analogue Scales (VAS) for severity of Raynaud's phenomenon and of digital ulcerations and Health Assessment Questionnaire (HAQ) disease index. Clinical data and blood samples, including complete blood count (CBC) and liver function tests (LFT) collected at inclusion and each visit thereafter, were used for treatment monitoring.

Baseline data and for each visit thereafter were collected prospectively. For each patient, data regarding demographics, SSc manifestations, the presence of specific autoantibodies, capillaroscopy patterns, date of bosentan treatment initiation and of 6-month and thereafter yearly visits, as well as blood tests results, were collected. Data regarding the number of DUs, VAS assessing severity of both Raynaud's phenomenon and DUs, HAQ disease index [13, 21] at initiation of bosentan and at follow-up visits were also recorded. Systemic sclerosis was classified as having diffuse (dcSSc) or limited cutaneous (lcSSc) involvement according to the classification described by LeRoy *et al.* [18].

Statistical analysis

The statistical analysis was performed using the IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). Data are presented as median, interquartile range (IQR). The Wilcoxon Signed Rank test for paired samples was used to compare clinical and laboratory data at baseline, 6 months and thereafter yearly follow-ups for up to 6 years. *p*-values < 0.05 were considered statistically significant.

All patients included in the study gave their written informed consent enabling the Centre to use their de-identified medical data in research projects. The study was approved by the local ethics committee.

Results and Discussion

Baseline characteristics of the patients are included in Table I.

Table I
Characteristics of the 49 patients at baseline

Baseline variables	Value
Gender (female)	39 (79.6)
dcSSc subset	28 (57.1)
Modified Rodnan skin score, median (IQR)	6 (9)
Age (years), median (IQR)	49.0 (18.0)
Disease duration (years), median (IQR)	9.0 (8.0)
Musculoskeletal involvement	41 (83.7)
Interstitial lung disease	35 (71.4)
Calcium-channel blockers	42 (85.7)
Low-dose Aspirin®	41 (83.7)
Prostanoids	14 (28.6)
Number of active DUs, median (IQR)	4 (4)
VAS DUs, median (IQR)	9 (3)
VAS Raynaud's phenomenon, median (IQR)	8 (2)
HAQ, median (IQR)	1.75 (1)
Anti-topoisomerase I antibodies	31 (63.3)

Values N (%) unless otherwise specified; dcSSc = diffuse cutaneous; DU = digital ulcers; HAQ = scleroderma health assessment questionnaire; VAS = Visual Analogue Scale

Briefly, 49 patients with SSc, among which 39 women, with a median (IQR) age of 49.0 (18.0) years old and disease duration of 9.0 (8.0) years, were included. The median (IQR) follow-up duration until last visit or dropout was 25 (43) months, min - max 4.7 - 75.1 months. The most frequent SSc manifestations apart from vascular involvement, were: gastrointestinal (n = 43), musculoskeletal (n = 41, including flexion ankylosis), interstitial lung disease (n = 35), cardiac involvement (n = 16). Forty-two (85.7%) patients underwent calcium channel blockers (CCB) treatment at the time of initiation of bosentan therapy, while the rest had a history of adverse events due to CCB administration which necessitated interruption. In addition, 41 (84.7%) patients were on low-doses of aspirin and 14 (28.6%) were administered prostanoids at the initiation of bosentan therapy.

The median (IQR) number of DUs at the treatment initiation time was 4.0 (4.0), range 1 - 28, while the VAS for Raynaud's phenomenon and for DUs was 8.0 (2.0), respectively 9.0 (3.0) and the median (IQR) HAQ was 1.75 (1.0). At the six-months follow-up, there was a significant reduction in the median (IQR) number of DUs to 0.0 (2.0), range 0 - 6, of the VAS for both Raynaud's phenomenon to 3.0 (3.0) and DUs

to 1.5 (2.75) and of the HAQ to 1.0 (1.25), which persisted at the twelve-months follow-up ($p < 0.001$ for all, by Wilcoxon Signed Rank test on paired data).

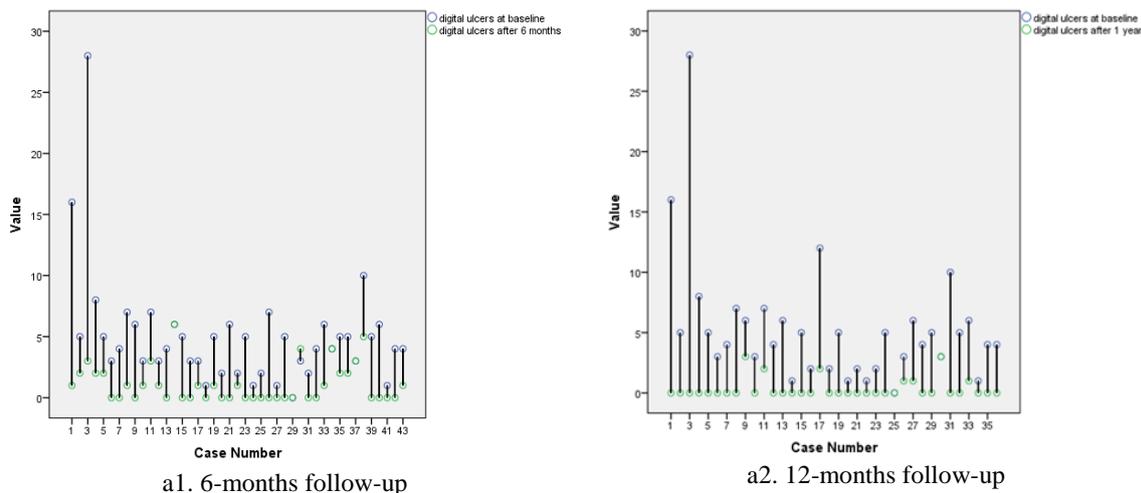
The evolution of the number of DUs throughout the follow-up duration is presented in Figure 2.

There were 9 patients who reached a treatment duration of 6 years; the significant improvement compared to baseline was maintained for the entire duration of follow-up for DUs, VAS Raynaud and VAS DUs (median (IQR) 0 (1), min - max 0 - 4, $p = 0.027$ for DUs, respectively 2.0 (1.0), $p = 0.034$ for VAS Raynaud's and 2.0 (1.5), $p = 0.038$ for VAS DUs), but with a gradual loss in meaningful differences of HAQ values compared to baseline (HAQ 0.63 (0.78), $p = 0.180$). mRSS had a tendency to decrease in patients with a longer treatment duration, but no clinically significant correlations between the mRSS and treatment duration were found.

There were 22 discontinuations of treatment: eight cases of elevation of aminotransferases more than three-fold the upper limit of normal values which required permanent interruption of bosentan treatment, one patient temporarily stopped the treatment in preparation of a pregnancy and resumed the administration of bosentan at 4 months after giving birth because of DUs reoccurrence, and three patients stopped the treatment for subjective reasons (anxiety, nausea and fatigue). In addition, there were five deaths due to complications of SSc, but not related to bosentan treatment, and five patients did not accomplish the follow-ups (no deaths among them), two of which continued the treatment in another medical centre. In two cases, the dose was reduced to 62.5 mg b.i.d because of persistent mild anaemia and, respectively, diarrhoea; the reduced dose was well tolerated. Three patients had known drug allergies, but none of them developed any allergic reaction to bosentan.

SSc-associated DUs are a debilitating vascular complication, with a major impact on the quality of life. The efficacy of bosentan in preventing the occurrence of new DUs has been demonstrated by two randomized clinical trials (RCTs), Randomized, double-blind, Placebo controlled study with bosentan on healing and prevention of Ischemic Digital ulcers in patients with systemic Sclerosis 1 and 2 (RAPIDS-1 and RAPIDS-2), which included 122, respectively 188 patients with SSc and at least one digital ulceration at baseline [15, 19], followed-up for 16, respectively 24 weeks. Another study performed on 30 SSc patients undergoing treatment with bosentan for a longer period of 24 months reported similar efficacy on preventing new DUs development and had no effect in the healing process of the pre-existent ones [16].

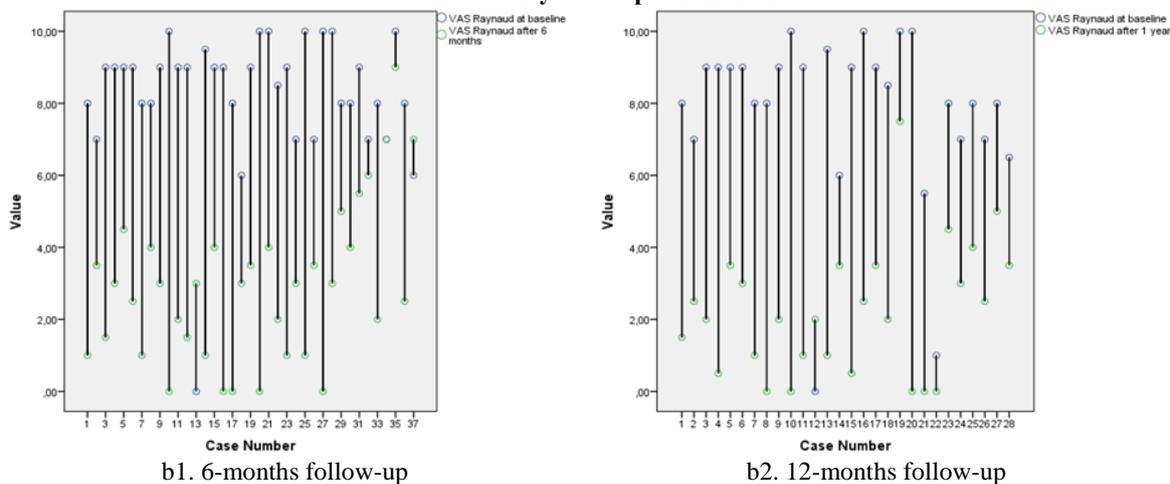
a. Number of DUs



a1. 6-months follow-up

a2. 12-months follow-up

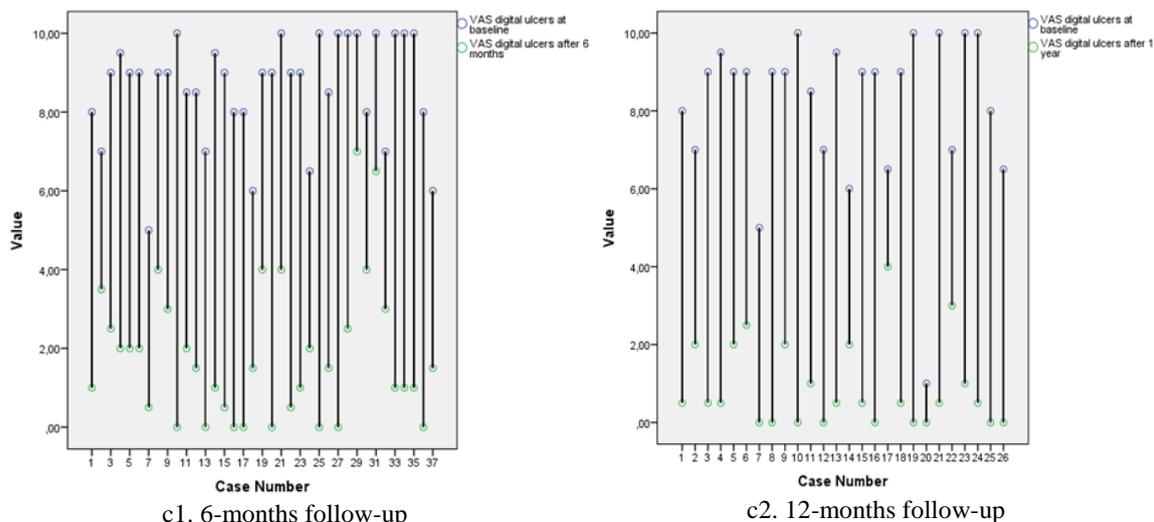
b. VAS for Raynaud's phenomenon



b1. 6-months follow-up

b2. 12-months follow-up

c. VAS for DUs



c1. 6-months follow-up

c2. 12-months follow-up

Figure 1.

Evolution at the 6-months and, respectively, 12-months follow-ups of the number of DUs, VAS for Raynaud's phenomenon and VAS for DUs [13, 21]

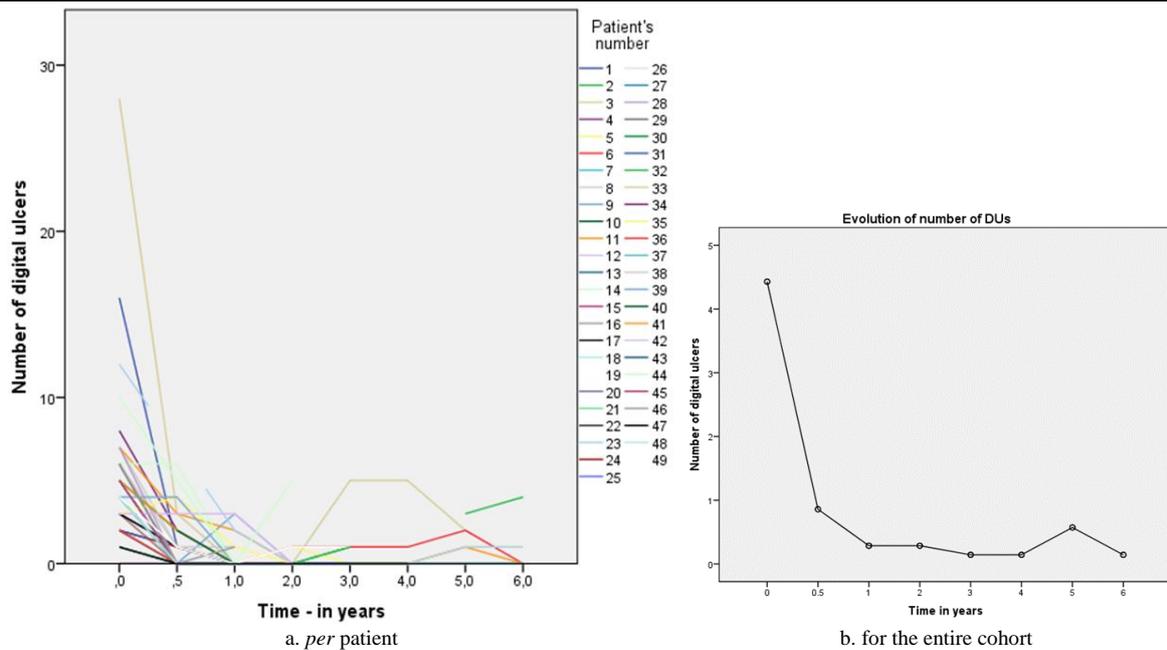


Figure 2.
Evolution of the number of DUs during the treatment with bosentan

In this prospective observational study, we followed up 49 SSc patients receiving bosentan for up to 6 years, focusing on the long-term efficacy and tolerability. In our cohort, the median (IQR) number of DUs significantly decreased at 6-months follow-up and has remained persistently low throughout the duration of treatment up to 6-years follow-up, confirming its long-term efficacy. This is the first study in patients with SSc from Romania to confirm persistent long-term effect of bosentan on preventing new DUs since being introduced in our country in 2014. The efficacy of bosentan on healing SSc-related DUs was surveyed in several studies [7, 8, 30], but no superiority over *placebo* was found, thus we did not investigate it further in our study.

Moreover, we evaluated the effect of bosentan on functional disability and vasculopathy-related quality of life of patients with SSc. We found a significant reduction of the burden of Raynaud's phenomenon and DU-related symptoms, expressed by a decrease in VAS scores for Raynaud's and DUs at 6-months follow-up, which persisted during the whole 6-years follow-up duration. There was also an initial significant decrease of the HAQ disease index compared to baseline, but this effect was lost after 2 years of treatment, which is more likely a consequence of several other causes of hand impairment such as increased severity of joint contractures [35], as the number of DUs remained low. These findings confirm the results of other studies [27], but which had shorter follow-up duration.

Over half of the patients in our cohort had dcSSc and over 70% of patients had a high prevalence of ILD or musculoskeletal involvement, confirming similar

reports that DUs are more prevalent in more severe diseases [24]. There have been reports that bosentan reverses the pro-fibrotic phenotype of SSc dermal fibroblasts in bleomycin-treated mice [2], however in human patients no improvement of skin fibrosis was observed after the treatment with bosentan [7]. We also did not find any significant associations between the mRSS and treatment duration in our cohort, but our cohort included patients with long disease duration, when there is a natural evolution of decrease in the mRSS, so no meaningful conclusions can be drawn in this regard.

Bosentan had an overall long-term good tolerability and determined few adverse events. The most notable adverse event encountered in our study was represented by elevated aminotransferases levels of more than three times the upper normal limit in approximately 16% of the patients, which implied permanent discontinuation of bosentan, as required by the national protocol. These results are consistent with data provided by the RAPIDS-1 and RAPIDS-2 trials, which noted hepatic cytolysis in 14% and 10.5% of patients undergoing treatment with bosentan vs. patients in the *placebo* group [3].

Our study has several limitations, including not adjusting for concomitant use of other vasoactive agents, such as intravenous iloprost or PDE-5 inhibitors, but their usage was limited in our cohort due to not being reimbursed by the national health insurance system. Furthermore, our study only assessed finger pulp DUs, as these are considered to have an ischemic cause. However, there is emerging evidence suggesting that all ulcers in SSc patients could have an ischemic

component and potentially respond to vasoactive therapy [9].

Conclusions

The results of the study suggest that bosentan is a long-term effective treatment in reducing the number of new DUs and improving hands function and vasculopathy-related quality of life and is generally well tolerated and safe.

Conflict of interest

The authors declare no conflict of interest.

References

1. Abraham S, Steen V, Optimal management of digital ulcers in systemic sclerosis. *Ther Clin Risk Manag.*, 2015; 11: 939-947.
2. Akamata K, Asano Y, Aozasa N, Noda S, Taniguchi T, Takahashi T, Ichimura Y, Toyama T, Sato S, Bosentan reverses the pro-fibrotic phenotype of systemic sclerosis dermal fibroblasts *via* increasing DNA binding ability of transcription factor Flil. *Arthritis Res Ther.*, 2014; 16(2): R86: 1-14.
3. Arefiev K, Fiorentino DF, Chung L, Endothelin receptor antagonists for the treatment of Raynaud's phenomenon and digital ulcers in systemic sclerosis. *Int J Rheumatol.*, 2011; 2011: 2017887: 1-7.
4. Avouac J, Riemekasten G, Meune C, Ruiz B, Kahan A, Allanore Y, Autoantibodies against endothelin 1 Type A receptor are strong predictors of digital ulcers in systemic sclerosis. *J Rheumatol.*, 2015; 42(10): 1801-1807.
5. CNAS/MS 1301/500/2008, List of therapeutic protocols approved by Order of Ministry of Health with the modifications and late updates, republished 03.09.2018, (available in Romanian).
6. Cutolo M, Soldano S, Smith V, Pathophysiology of systemic sclerosis: current understanding and new insights. *Expert Rev Clin Immunol.*, 2019; 15(7): 753-764.
7. De Cata A, Inglese M, Molinaro F, De Cosmo S, Rubino R, Bernal M, Mazzocchi G, Digital ulcers in scleroderma patients: A retrospective observational study. *Int J Immunopathol Pharmacol.*, 2016; 29(2): 180-187.
8. García de la Peña Lefebvre P, Nishishinya MB, Pereda CA, Loza E, Sifuentes Giraldo WA, Román Ivorra JA, Carreira P, Rúa-Figueroa I, Pego-Reigosa JM, Muñoz-Fernández S, Efficacy of Raynaud's phenomenon and digital ulcer pharmacological treatment in systemic sclerosis patients: a systematic literature review. *Rheumatol Int.*, 2015; 35(9): 1447-1459).
9. Hughes M, Alcacer-Pitarch B, Gheorghiu AM, Praino E, Sandler RD, Tavor Y, Bruni C, Matucci-Cerinic M, Digital ulcer debridement in systemic sclerosis: a systematic literature review. *Clin Rheumatol.*, 2020; 39(3): 805-811.
10. Hughes M, Allanore Y, Chung L, Pauling JD, Denton CP, Matucci-Cerinic M, Raynaud phenomenon and digital ulcers in systemic sclerosis. *Nat Rev Rheumatol.*, 2020; 16(4): 208-221.
11. Hughes M, Murray A, Denton CP, Herrick AL, Should all digital ulcers be included in future clinical trials of systemic sclerosis-related digital vasculopathy?. *Med Hypotheses*, 2018; 116: 101-104.
12. Jimenez SA, Derk CT, Following the molecular pathways toward an understanding of the pathogenesis of systemic sclerosis. *Ann Intern Med.*, 2004; 140(1): 37-50.
13. Johnson SR, Hawker GA, Davis AM, The health assessment questionnaire disability index and scleroderma health assessment questionnaire in scleroderma trials: An evaluation of their measurement properties. *Arthritis Rheum.*, 2005; 53(2): 256-262.
14. Kahaleh B, Meyer O, Scorza R, Assessment of vascular involvement. *Clin Exp Rheumatol.*, 2003; 21(3 Suppl 29): S9-S14.
15. Korn JH, Mayes M, Matucci Cerinic M, Rainisio M, Pope J, Hachulla E, Rich E, Carpentier P, Molitor J, Seibold JR, Hsu V, Guillevin L, Chatterjee S, Peter HH, Coppock J, Herrick A, Merkel PA, Simms R, Denton CP, Furst D, Nguyen N, Gaitonde M, Black C, Digital ulcers in systemic sclerosis: Prevention by treatment with bosentan, an oral endothelin receptor antagonist. *Arthritis Rheum.*, 2004; 50(12): 3985-3993.
16. Küçükşahin O, Yildizgören MT, Gerece DM, Maraş Y, Erten Ş, Bosentan for digital ulcers in patients with systemic sclerosis: Single center experience. *Arch Rheumatol.*, 2016; 31(3): 229-233.
17. Lafyatis R, Varga J, Etiology and pathogenesis of systemic sclerosis. In Hochberg M, 7th Edition, Rheumatology, 2018; Vol. 2: 1249-1262.
18. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, Rowell N, Wollheim F, Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol.*, 1988; 15(2): 202-205.
19. Matucci-Cerinic M, Denton C P, Furst DE, Mayes MD, Hsu VM, Carpentier P, Wigley FM, Black CM, Fessler BJ, Merke PA, Pope JE, Weiss NJ, Doyle MK, Hellmich B, Medsger TA, Morganti A, Kramer F, Korn JH, Seibold JR, Bosentan treatment of digital ulcers related to systemic sclerosis: Results from the RAPIDS-2 randomised, double-blind, placebo-controlled trial. *Ann Rheum Dis.*, 2011; 70(1): 32-38.
20. Matucci-Cerinic M, Krieg T, Guillevin L, Schwierin B, Rosenberg D, Cornelisse P, Denton CP, Elucidating the burden of recurrent and chronic digital ulcers in systemic sclerosis: Long-term results from the DUO Registry. *Ann Rheum Dis.*, 2016; 75(10): 1770-1776.
21. Merkel PA, Herlyn K, Martin RW, Anderson JJ, Mayes MD, Bell P, Korn JH, Simms RW, Csuka ME, Medsger TA, Rothfield NF, Ellman MH, Collier DH, Weinstein A, Furst DE, Jiménez SA, White B, Seibold JR, Wigley FM, Measuring disease activity and functional status in patients with scleroderma and Raynaud's phenomenon. *Arthritis Rheum.*, 2002; 46(9): 2410-2420.
22. Mihai C, Distler O, Gheorghiu AM, Constantin PI, Dobrota R, Jordan S, Smith V, Hachulla E, Henes J, Siegert E, Vettori S, Müller-Ladner U, Matucci Cerinic M, Allanore Y, EUSTAR collaborators, Incidence and risk factors for gangrene in patients with systemic sclerosis from the EUSTAR cohort. *Rheumatology (Oxford)*, 2020; 59(8): 2016-2023.

23. Mihai C, Landewé R, van der Heijde D, Walker UA, Constantin PI, Gherghe AM, Ionescu R, Rednic S, Allanore Y, Avouac J, Czirják L, Hachulla E, Riemekasten G, Cozzi F, Airò P, Cutolo M, Mueller-Ladner U, Matucci-Cerinic M, EUSTAR co-authors, Digital ulcers predict a worse disease course in patients with systemic sclerosis. *Ann Rheum Dis.*, 2016; 75(4): 681-686.
24. Morrisroe K, Stevens W, Sahhar J, Ngian GS, Ferdowsi N, Hill CL, Roddy J, Walker J, Proudman S, Nikpour M, Digital ulcers in systemic sclerosis: Their epidemiology, clinical characteristics, and associated clinical and economic burden. *Arthritis Res Ther.*, 2019; 21(1): 299: 1-12.
25. Mouthon L, Carpentier PH, Lok C, Clerson P, Gressin V, Hachulla E, Bézné A, Diot E, Khau Van Kien A, Jegu P, Agard C, Duval-Modeste AB, Sparsa A, Puzenat E, Richard MA, ECLIPSE Study Investigators, Ischemic digital ulcers affect hand disability and pain in systemic sclerosis. *J Rheumatol.*, 2014; 41(7): 1317-1323.
26. Pattanaik D, Brown M, Postlethwaite AE, Vascular involvement in systemic sclerosis (scleroderma). *J Inflamm Res.*, 2011; 4: 105-125.
27. Rezus E, Burlui A, Gafton B, Stratulat T, Zota G, Cardoneanu A, Rezus C, A patient-centered approach to the burden of symptoms in patients with scleroderma treated with Bosentan: A prospective single-center observational study. *Exp Ther Med.*, 2020; 19(3): 1739-1746.
28. Steen V, Denton CP, Pope JE, Matucci-Cerinic M, Digital ulcers: overt vascular disease in systemic sclerosis. *Rheumatology (Oxford)*, 2009; 48(Suppl 3): 19-24.
29. Tilinca MC, Merlan I, Salcudean A, Tilea I, Nemes-Nagy E, Oxidative stress and cytokines' involvement in the occurrence and progression of diabetic complications in the COVID-19 pandemic context. *Farmacia*, 2021; 69(4): 635-641.
30. Tingey T, Shu J, Smuczek J, Pope J, Meta-analysis of healing and prevention of digital ulcers in systemic sclerosis. *Arthritis Care Res (Hoboken)*, 2013; 65(9): 1460-1471.
31. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, Matucci-Cerinic M, Naden RP, Medsger TA Jr, Carreira PE, Riemekasten G, Clements PJ, Denton CP, Distler O, Allanore Y, Furst DE, Gabrielli A, Mayes MD, van Laar JM, Seibold JR, Czirjak L, Steen VD, Inanc M, Kowal-Bielecka O, Müller-Ladner U, Valentini G, Veale DJ, Vonk MC, Walker UA, Chung L, Collier DH, Csuka ME, Fessler BJ, Guiducci S, Herrick A, Hsu VM, Jimenez S, Kahaleh B, Merkel PA, Sierakowski S, Silver RM, Simms RW, Varga J, Pope JE, 2013 classification criteria for systemic sclerosis: An American college of rheumatology/European league against rheumatism collaborative initiative. *Ann Rheum Dis.*, 2013; 72(11): 1747-1755.
32. Walker UA, Tyndall A, Czirják L, Denton C, Farge-Bancel D, Kowal-Bielecka O, Müller-Ladner U, Bocelli-Tyndall C, Matucci-Cerinic M, Clinical risk assessment of organ manifestations in systemic sclerosis: A report from the EULAR Scleroderma Trials and Research group database. *Ann Rheum Dis.*, 2007; 66(6): 754-763.
33. Yakimova B, Mateeva P, Kardaleva P, Stoineva I, Todorova P, Zamfirova R, Yanev S, *In vitro* and *ex vivo* studies on angiotensin-I converting enzyme (Ace) inhibitory activity of short synthetic peptides. *Farmacia*, 2021; 69(2): 307-313.
34. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T, A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, 1988; 332(6163): 411-415.
35. Young A, Namas R, Dodge C, Khanna D, Hand Impairment in Systemic Sclerosis: Various Manifestations and Currently Available Treatment. *Curr Treatm Opt Rheumatol.*, 2016; 2(3): 252-269.