Difference in Serum Endostatin Levels in Diabetic Patients with Critical Limb Ischemia Treated by Autologous Cell Therapy or Percutaneous Transluminal Angioplasty

Andrea Nemcova¹, Alexandra Jirkovska¹, Michal Dubsky¹, Libor Kolesar², Robert Bem¹, Vladimira Fejfarova¹, Anna Pysna¹, Veronika Woskova¹, Jelena Skibova¹, and Edward B. Jude³

Abstract
The aim of this study was to compare the serum levels of the anti-angiogenic factor endostatin (S-endostatin) as a potential marker of vasculogenesis after autologous cell therapy (ACT) versus percutaneous transluminal angioplasty (PTA) in diabetic patients with critical limb ischemia (CLI). A total of 25 diabetic patients with CLI treated in our foot clinic during the period 2008–2014 with ACT generating potential vasculogenesis were consecutively included in the study; 14 diabetic patients with CLI who underwent PTA during the same period were included in a control group in which no vasculogenesis had occurred. S-endostatin was measured before revascularization and at 1, 3, and 6 months after the procedure. The effect of ACT and PTA on tissue ischemia was confirmed by transcutaneous oxygen pressure (TcPO2) measurement at the same intervals. While S-endostatin levels increased significantly at 1 and 3 months after ACT (both P < 0.001), no significant change of S-endostatin after PTA was observed. Elevation of S-endostatin levels significantly correlated with an increase in TcPO2 at 1 month after ACT (r = 0.557; P < 0.001). Our study showed that endostatin might be a potential marker of vasculogenesis because of its significant increase after ACT in diabetic patients with CLI in contrast to those undergoing PTA. This increase may be a sign of a protective feedback mechanism of this anti-angiogenic factor.

Keywords
angiogenic factors, autologous cell therapy, critical limb ischemia, endostatin

Introduction
Critical limb ischemia (CLI) represents the end stage of peripheral arterial disease (PAD) and portends a high rate of limb loss along with patient mortality rates¹. The diagnosis and therapy of CLI in patients with diabetes is challenging because they frequently lack typical symptoms due to peripheral neuropathy, and most of the lesions affect infrapopliteal arteries². Traditionally, standard methods in the treatment of CLI have been open bypass surgery or endovascular interventions, such as percutaneous transluminal angioplasty (PTA) or stenting³. However, about 30% of CLI patients are not eligible for standard revascularization due to excessive operative risk or unfavorable vascular involvement⁴. These patients with no-option CLI might obtain therapeutic benefit from autologous cell therapy (ACT) as an alternative therapeutic modality for CLI studied in clinical trials⁵,⁶. Previous studies have shown that therapy using autologous cells injected into the affected lower limb was well tolerated and effective for achievement of better clinical outcomes⁷,⁸.

ACT is based on cell suspension, which consists of heterogeneous mononuclear cells containing a progenitor cell
population harvested from bone marrow (bone marrow-derived mononuclear cells; BMMNCs) or peripheral blood progenitor cells (PBPCs) separated from peripheral blood after stimulation by granulocyte colony-stimulating factor (G-CSF)\textsuperscript{9}. According to previous studies\textsuperscript{10,11}, the cell population characterized by CD34\textsuperscript{+} surface marker is probably the most important cell type for vascular regeneration and is mainly composed of hematopoietic stem cells with a subset of endothelial progenitor cells (EPCs)\textsuperscript{12,13}. EPCs are capable of migrating from the bone marrow and differentiate into mature endothelium in the areas of ischemia\textsuperscript{14}.

While the exact mechanism of action of injected autologous cells at tissue level remains unclear, their involvement in vasculogenesis is assumed. Two possible mechanisms of vasculogenesis – angiogenesis and arteriogenesis – have been suggested\textsuperscript{15}. Unlike angiogenesis, which is induced by hypoxia and results in the growth of new capillaries, arteriogenesis is induced by physical forces, mainly increased fluid shear stress that leads to remodeling and maturation of pre-existing collaterals\textsuperscript{16,17}. One of the possible explanations for the mechanism of action of ACT is that these injected cells can directly contribute to vasculogenesis by being incorporated into nascent vessels; alternatively, they can release growth factors that affect endogenous vascular cells, or directly instruct host cells to secrete angiogenic growth factors\textsuperscript{18}.

The release of pro- and anti-angiogenic factors is a possible modulator of vasculogenesis\textsuperscript{19}. The most important pro-angiogenic factors include vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)\textsuperscript{20}, angiopoietin-1, platelet-derived growth factors (PDGF-AA, PDGF-BB), or hypoxia-inducible factor (HIF-1\textalpha)\textsuperscript{21}, whereas the main anti-angiogenic factors are represented by endostatin, angiostatin, arresten, canstatin, tumstatin, or thrombospondins\textsuperscript{22}.

Endostatin, one of the most potent inhibitors of angiogenesis, is cleaved from collagen XVIII, which is a component of vascular basement membranes\textsuperscript{23}. Endostatin is able to inhibit angiogenesis by restricting endothelial cell proliferation and migration, and by inducing endothelial cell apoptosis\textsuperscript{24}. There has been extensive research focused on the potential clinical uses for endostatin, such as an anticancer drug or in the treatment of inflammatory diseases, diabetic retinopathy, or obesity. It is also used as a prognostic biomarker or kidney disease marker\textsuperscript{25–26}. On the other hand, the origin of circulating levels of endostatin is still not known and it is unclear what the circulating endostatin levels actually reflect\textsuperscript{27}.

The standard technique for non-invasive measurement of limb ischemia is transcutaneous oxygen pressure (TcPO\textsubscript{2}) as a main parameter for assessing the therapeutic effect in most studies focused on cell therapy of CLI\textsuperscript{6,17,28}. In our previous study, we documented the effect of ACT by a significant increase in TcPO\textsubscript{2} levels at 12-month follow-up\textsuperscript{29}.

Previously, we assessed the serum levels of pro-angiogenic factors as markers of systemic side effects of ACT\textsuperscript{19}. We observed no significant increase in the serum levels of pro-angiogenic factors and found no signs of distal systemic vasculogenesis on fundoscopic examination of the eye. Surprisingly, we did document a significant increase in serum endostatin levels after ACT as a finding to suggest lines of further research based on a hypothesis that the anti-angiogenic factor endostatin could potentially reflect vasculogenesis after ACT.

The aim of our present study was to evaluate the serum levels of the anti-angiogenic factor endostatin in diabetic patients with CLI treated by ACT versus PTA, to assess the association of endostatin with the number of injected angiogenic precursor cells (CD34\textsuperscript{+}), and to compare the kinetics of endostatin with changes in TcPO\textsubscript{2} potentially reflecting improvement of ischemia after ACT.

**Materials and Methods**

A total of 25 diabetic patients with no-option CLI treated by cell therapy in our foot clinic during the period 2008–2014 were consecutively included in the study; 14 diabetic patients with CLI undergoing PTA in our foot clinic over the same period of time were included in a control group.

The presence of CLI was defined by TcPO\textsubscript{2} < 30 mmHg, objectively proven below-the-knee stenosis or obliteration on the angiogram, non-healing ulcer, or ischemic pain consistent with the Trans-Atlantic Inter-Society Consensus II criteria for CLI\textsuperscript{30}. Patients meeting the inclusion criteria while ineligible for standard revascularization were treated by ACT; those included in the control group underwent PTA. The severity of angiographic findings was assessed in all patients using the Graziani classification\textsuperscript{31}. Patients treated by ACT followed a protocol that included detailed oncological, hematological, and angiographic screening as described in detail in our previous study\textsuperscript{29}.

Exclusion criteria for ACT were diagnosed neoplasm of any organ, severe hematological abnormality, deep vein thrombosis in the past 6 months, non-treated proliferative retinopathy, severe limb edema, or severe foot infection with high risk of major amputation. The study was approved by the local ethics committee (Joint Ethics Committee of the Institute for Clinical and Experimental Medicine and Thon Mayer Hospital), and all patients gave written informed consent.

Using the Jamshidi technique, bone marrow was harvested from both sides of the iliac crest in the operating theater, with BMMNCs subsequently separated using the Smart PreP2 system (Harvest Technologies Corporation, Plymouth, MA, USA) or sedimented using sucinated gelatin (Gelofusin; B. Braun, Melsungen, Germany). Final cell suspension of 40–70 ml was injected into the muscles of the affected lower limb in a series of 40–50 punctures of approximately 1–2 ml each, and also to the wound edges.

TcPO\textsubscript{2} measurement was performed by standardized method on a TCM400 monitor (Radiometer Medical ApS,
Aarhus, Denmark) before revascularization and at 1, 3, and 6 months after the procedure by a foot care specialist.

The serum levels of endostatin (S-endostatin) were measured before and at 1, 3, and 6 months after revascularization either by ACT or PTA, and were compared with changes in TcPO2 at the same intervals.

**Endostatin Analysis**

Serum samples were stored at –80°C until assayed. These samples were diluted 1:4; endostatin was assessed using the commercial Fluorokine MAP Human Angiogenesis Custom Premix Kit A (R&D Systems, Minnesota, MN, USA) and Luminex device (Luminex Corporation, Austin, TX, USA). The minimum detection level for endostatin was 123.5 pg/ml. Diluted serum samples (50 μl) were mixed with an analyte-specific antibody-coated microparticle and incubated for 3 h at room temperature with gentle, continuous agitation. To this was added a detection antibody for endostatin, then it was incubated again at room temperature for 1 h on a horizontal vortex device. Next, phycoerythrin-conjugated streptavidin (from the Fluorokine Kit) was added and incubated for a further 30 min at room temperature on the vortex. The samples were then immediately measured in the Luminex device. The sample results were calculated by interpolation from particular standard curves.

**CD34+ Cell Analysis**

Fluorescence-activated cell sorting (FACS) analysis was done with a FC500 system (Beckman Coulter, Brea, CA, USA), and the number of injected CD34+ cells in the BMMNC suspension calculated based on the detected CD34+ concentration and the volume of injected cell suspension.

All data were expressed as mean ± SD. Wilcoxon pair test, Mann–Whitney test, and Spearman correlation coefficient were used for statistical analysis.

**Results**

Characteristics of diabetic patients in both groups are summarized in Table 1. We did not observe any significant differences between the study groups except for lower baseline TcPO2 levels and more severe angiographic finding according to the Graziani classification for ACT-treated patients. Patients in the two groups did not differ significantly in baseline endostatin levels.

While the serum levels of the angiogenic inhibitor endostatin were significantly increased at 1 and 3 months after ACT (from 131.6 ± 88.3 to 159.7 ± 113.1 and 173.1 ± 119.3 ng/ml, respectively; both P < 0.001), no significant changes in S-endostatin were seen after PTA (Figure 1). Figure 3 shows an increase in TcPO2 after ACT in comparison to changes in S-endostatin; there was a continuing significant increase in TcPO2 levels at 3 and 6 months after ACT, while the levels of S-endostatin showed a significant increase at 1 and 3 months to return to baseline at 6 months post-ACT. While observing a significant correlation between S-endostatin and TcPO2 after ACT, no correlation between S-endostatin and TcPO2 after PTA was seen.

### Table 1. Basic Characteristics of Patients.

|                          | ACT group | PTA group | P   |
|--------------------------|-----------|-----------|-----|
| Age (years)              | 65 ± 8.8  | 63.7 ± 7.5| NS  |
| Gender (% men)           | 88        | 85.7      | NS  |
| Type 2 diabetes (%)      | 84        | 71.4      | NS  |
| Diabetes duration (years)| 21.3 ± 13.4| 28 ± 12   | NS  |
| HbA1c (mmol/mol)         | 59.2 ± 13.5| 63.6 ± 13.6| NS  |
| TcPO2 (mmHg)             | 18 ± 10.4 | 27.5 ± 15.2| 0.001|
| Graziani classification  | 5.4 ± 1.0 | 3.4 ± 0.8 | 0.001|
| (mean ± SD)              |           |           |     |
| Ischemic heart disease (%)| 56        | 21.4      | 0.03|
| Hypertension (%)         | 88        | 78.6      | NS  |
| Chronic renal failure –  | 12        | 7.1       | NS  |
| dialysis (%)             |           |           |     |
| Smoking (current + ex %) | 48        | 42.9      | NS  |
| Baseline endostatin levels (ng/ml) | 131.6 ± 88.3 | 139.2 ± 34.7 | NS   |
| Number of injected CD34+ cells (x10^6) | 12.9 ± 10.8 | – |     |

ACT, autologous cell therapy; PTA, percutaneous transluminal angioplasty; SD, standard deviation; TcPO2, transcutaneous oxygen pressure.

![Figure 1. Comparison of S-endostatin levels between patients after ACT and PTA. S-endostatin increased significantly at 1 and 3 months after ACT (**p < 0.001**) in contrast to no significant changes of S-endostatin after PTA. ACT, autologous cell therapy; PTA, percutaneous transluminal angioplasty.](image-url)
The mean number of CD34+ cells in the suspension injected to the lower limb was 12.9 ± 10.8 × 10^6; there was no significant association between the number of injected CD34+ cells and S-endostatin levels.

Discussion

Our study showed a significant increase in the serum levels of the endogenous angiogenesis inhibitor endostatin at 1 and 3 months after ACT, in contrast to statistically non-significant changes in this factor after PTA. These results may support our hypothesis that the increase in endostatin could be a compensatory mechanism during transient vasculogenesis after ACT through a negative feedback loop, in contrast to PTA whereby no vasculogenesis occurs. PTA re-establishes the patency of an artery by transluminal or subintimal procedures without a direct impact on new vessel formation.

The mechanism of the anti-angiogenic activity of endostatin was described previously. Endostatin shows potent inhibition of endothelial cell migration and proliferation, and induction of their apoptosis; it is able to inhibit the VEGF-induced mobilization of EPCs into the circulation, thereby suppressing angiogenesis. Another proposed mechanism of the anti-angiogenic action of endostatin is inhibition of matrix metalloproteinases that facilitate endothelial cell migration and invasion during angiogenesis by proteolytic extracellular matrix degradation. These effects of endostatin are most likely due to its binding to cell surface receptors and influencing a series of intracellular signaling cascades through the interaction with a number of proteins such as integrins, troponyosin, glypicans, or laminin that mediate the anti-endothelial functions of endostatin. Folkmann et al. suggested that only “pathogenic-derived” angiogenesis usually involves a signal pathway through integrins, which are directly affected by endostatin in contrast to “physiological” angiogenesis that occurs during the wound-healing process.

Similar to our study, Xue et al. investigated early changes in the expression of endostatin and VEGF in patients after acute ischemic stroke. They found that serum endostatin levels tended to increase significantly later than those of VEGF, and remained higher until the end of the observation. This asynchronous expression of VEGF and endostatin indicates that VEGF may possibly play an important role in the early stages of cerebral ischemia through triggering endogenous angiogenesis and increasing vascular permeability, whereas endostatin may represent protective feedback mechanisms. These results are in agreement with possible endostatin feedback regulation of vasculogenesis after ACT of CLI in diabetic patients.
We did not observe any correlation between the number of injected CD34+ cells and serum levels of endostatin. A supposed clinical effect of ACT is probably mediated by the interaction of cells in cell suspension and influenced by the viability of these cells rather than by the total number of injected CD34+ cells. Similar to our finding, no relation between the number of injected CD34+ cells and clinical effect has been confirmed in previous trials: Kawamoto et al., using three different doses of CD34+ cells per kilogram in patients with CLI, observed no significant dose–response effect in terms of the clinical effect as assessed by TcPO2. Likewise, Dong et al., using purified CD34+ cells in CLI patients, did not report a correlation between improvement of clinical outcomes and low, medium, or high doses of injected CD34+ cells. On the other hand, Madaric et al. investigated factors associated with clinical benefits of ACT in patients with no-option CLI and discovered that the number of injected CD34+ cells was an independent predictor of limb salvage and wound healing.

The clinical effect both of ACT and PTA was confirmed by a significant increase in TcPO2 levels from baseline at all intervals during follow up. A significant correlation between endostatin and levels of TcPO2 was observed only at 1 month after ACT because of the different kinetics of endostatin and changes of TcPO2. A temporary significant increase in endostatin at 1 and 3 months post-ACT may reflect vasculogenesis with a subsequent decrease in endostatin after completion of the new-vessel-formation process. Kinnaird et al. published that the process of remodeling of a completely patent three-layered collateral artery restores blood flow within 4–6 weeks. Unlike the temporary increase in endostatin, increased TcPO2 levels persist because the ACT-mediated improvement of microcirculation increases tissue oxygenation for a longer time as documented in our previous study.

The increase in endostatin as a potential marker of new vasculogenesis in our present study seems to be in contrast to the results of several earlier studies in which elevated endostatin levels were associated with ischemia, not with revascularization. For example, Qin et al. reported that S-endostatin was significantly higher in patients with severe coronary artery disease and poor collateral circulation compared with patients with normal collaterals. The discrepancy of these results with our findings of a transient endostatin increase as a marker of vasculogenesis could be explained by different physiological pathways: In our study, the increase in endostatin was likely stimulated as a feedback reaction to ACT-induced new vasculogenesis in contrast to the increase in the levels of endostatin as a marker of poor collateral formation in Qin’s study. In another study, Golledge et al. reported that S-endostatin was elevated in men with symptoms of intermittent claudication compared to those without ischemic leg pain. The discrepancy between the above results and ours could again be explained by the different mechanisms causing the changes in endostatin levels.

The advantages of our study include its prospective follow up, well-defined inclusion criteria and methods used for assessment, and comparability of both groups of patients in terms of their demographic characteristics. The main limitation of our study is our inability to verify increased vasculogenesis after ACT by appropriate immunohistochemical techniques at tissue level due to the absence of such a method or unavailability of the necessary technologies. Another limitation of this study is its pilot design, with a limited number of patients in both groups.

Conclusion

In conclusion, our study showed that the anti-angiogenic factor endostatin, temporarily elevated after ACT, could reflect new vasculogenesis induced by this therapy in diabetic patients with CLI. A potentially protective feedback role of endostatin in ACT-mediated vasculogenesis poses a future research challenge.

Ethical Approval

This study was approved by the Joint Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital, Prague, Czech Republic.

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the Ethics Committee of the Institute for Clinical and Experimental Medicine and institutional review board’s approved protocols.

Statement of Informed Consent

All patients gave informed consent.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by Charles University in Prague, project GAUK 16415 and by the Ministry of Health of the Czech Republic, grant no. 16-27262A and by the project for Development of Research Organization 00023001 (IKEM, Prague, Czech Republic) – Institutional support.

References

1. Dua A, Lee CJ. Epidemiology of peripheral arterial disease and critical limb ischemia. Tech Vasc Interv Radiol. 2016;19(2):91–5.
2. Brownrigg JR, Schaper NC, Hinchliffe RJ. Diagnosis and assessment of peripheral arterial disease in the diabetic foot. Diabet Med. 2015;32(6):738–47.
3. Benitez E, Sumpio BJ, Chin J, Sumpio BE. Contemporary assessment of foot perfusion in patients with critical limb ischemia. Semin Vasc Surg. 2014;27(1):3–15.

Institutional support.
4. Compagna R, Amato B, Massa S, Amato M, Grande R, Butrico L, de Franciscis S, Serra R. Cell therapy in patients with critical limb ischemia. Stem Cells Int. 2015;2015:931420.

5. Huang P, Li S, Han M, Xiao Z, Yang R, Han ZC. Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. Diabetes Care. 2005;28(9):2155–60.

6. Fadini GP, Agostini C, Avogaro A. Autologous stem cell therapy for peripheral arterial disease meta-analysis and systematic review of the literature. Atherosclerosis. 2010;209(1):10–17.

7. Liu Y, Xu Y, Fang F, Zhang J, Guo L, Weng Z. Therapeutic efficacy of stem cell-based therapy in peripheral arterial disease: A meta-analysis. PLoS One. 2015;10(4):e0125032.

8. Ai M, Yan CF, Xia FC, Zhou SL, He J, Li CP. Safety and efficacy of cell-based therapy on critical limb ischemia: A meta-analysis. Cytotherapy. 2016;18(6):712–24.

9. Aghi M, Chiocca EA. Contribution of bone marrow-derived cells to blood vessels in ischemic tissues and tumors. Mol Ther. 2005;12(6):994–1005.

10. Li S, Zhou B, Han ZC. Therapeutic neovascularization by transplantation of mobilized peripheral blood mononuclear cells for limb ischemia. A comparison between CD34+ and CD34+ mononuclear cells. Thromb Haemost. 2006;95(2):301–11.

11. Mackie AR, Losordo DW. CD34-positive stem cells: In the treatment of heart and vascular disease in human beings. Tex Heart Inst J. 2011;38(5):474–85.

12. Fadini GP, Rigato M, Cappellari R, Bonora BM, Avogaro A. Long-term prediction of cardiovascular outcomes by circulating CD34+ and CD34+CD133+ stem cells in patients with type 2 diabetes. Diabetes Care. 2017;40(1):125–31.

13. Zubair AC, Malik S, Paulsen A, Ishikawa M, McCoy C, Adams PX, Amrani D, Costa M. Evaluation of mobilized peripheral blood CD34+ cells from patients with severe coronary artery disease as a source of endothelial progenitor cells. Cytotherapy. 2010;12(2):178–89.

14. Hristov M, Weber C. Progenitor cell trafficking in the vascular wall. J Thromb Haemost. 2009;7(Suppl 1):31–34.

15. Buschmann I, Schaper W. Arteriogenesis versus angiogenesis: Two mechanisms of vessel growth. News Physiol Sci. 1999;14:121–5.

16. Cooke JP, Losordo DW. Modulating the vascular response to limb ischemia: Angiogenic and cell therapies. Circ Res. 2015;116(9):1561–78.

17. Lawall H, Bramlage P, Amann B. Stem cell and progenitor cell therapy in peripheral artery disease. A critical appraisal. Thromb Haemost. 2010;103(4):696–709.

18. Aranguren XL, Verfaillie CM, Luttun A. Emerging hurdles in stem cell therapy for peripheral vascular disease. J Mol Med (Berl). 2009;87(1):3–16.

19. Dubsky M, Jirkovska A, Bem R, Fejfarova V, Varga M, Kolesar L, Pagacova L, Sykova E, Jude EB. Role of serum levels of angiogenic cytokines in assessment of angiogenesis after stem cell therapy of diabetic patients with critical limb ischemia. Cell Transplant. 2014;23(12):1517–23.

20. Di Vita G, Patti R, D’Agostino P, Arcoleo F, Caruso G, Arcara M, Davi V, Cillari E. Serum VEGF and B-FGF profiles after tension-free or conventional hernioplasty. Langenbecks Arch Surg. 2005;390(6):528–33.

21. Kimnaird T, Stabile E, Burnett MS, Epstein SE. Bone-marrow-derived cells for enhancing collateral development: Mechanisms, animal data, and clinical experiences. Circ Res. 2004;95(4):354–63.

22. Ribatti D. Endogenous inhibitors of angiogenesis: A historical review. Leuk Res. 2009;33(5):638–44.

23. Poluzzi C, Izzo RV, Schaefer L. Endostatin and endorepellin: A common route of action for similar antiangiostatic cancer agents. Adv Drug Deliv Rev. 2016;97:156–73.

24. Wallia A, Yang JF, Huang YH, Rosenblatt MI, Chang JH, Azar DT. Endostatin’s emerging roles in angiogenesis, lymphangiogenesis, disease, and clinical applications. Biochim Biophys Acta. 2015;1850(12):2422–38.

25. Wang H, Chen Y, Lu XA, Liu G, Fu Y, Luo Y. Endostatin prevents dietary-induced obesity by inhibiting adipogenesis and angiogenesis. Diabetes. 2015;64(7):2442–56.

26. Carlsson AC, Ostgren CJ, Lanne T, Larsson A, Nystrom FH, Arnlov J. The association between endostatin and kidney disease and mortality in patients with type 2 diabetes. Diabetes Metab. 2016;42(5):351–7.

27. Ruge T, Carlsson AC, Larsson A, Arnlov J. Endostatin: A promising biomarker in the cardiovascular continuum? Biomark Med. 2017;11(10):905–16.

28. Al Mheid I, Quyyumi AA. Cell therapy in peripheral arterial disease. Angiology. 2008;59(6):705–16.

29. Dubsky M, Jirkovska A, Bem R, Fejfarova V, Pagacova L, Nemcova A, Sixta B, Chlupac J, Peregrin JH, Sykova E, Jude EB. Comparison of the effect of stem cell therapy and percutaneous transluminal angioplasty on diabetic foot disease in patients with critical limb ischemia. Cytotherapy. 2014;16(12):1733–8.

30. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, Group TIW, Bell K, Caporossu J, Durand-Zaleski I, Konsor K, Lammer J, Liapis C, Novo S, Razavi M, Robbs J, Schaper N, Shigematsu H, Soperal M, White C, White J, Clement D, et al. Inter-society consensus for the management of peripheral arterial disease (TASC II). Eur J Vasc Endovasc Surg. 2007;33(Suppl 1):S1–75.

31. Graziani L, Silvestro A, Bertone V, Manara E, Andreini R, Sigala A, Mingardi R, De Giglio R. Vascular involvement in diabetic subjects with ischemic foot ulcer: A new morphologic categorization of disease severity. Eur J Vasc Endovasc Surg. 2007;33(4):453–60.

32. Schuch G, Heymach JV, Nomri M, Machluf M, Force J, Atala A, Eder JP Jr., Folkman J, Soker S. Endostatin inhibits the vascular endothelial growth factor-induced mobilization of endothelial progenitor cells. Cancer Res. 2003;63(23):8345–50.

33. Fu Y, Tang H, Huang Y, Song N, Luo Y. Unraveling the mysteries of endostatin. IUBMB Life. 2009;61(6):613–26.

34. Folkman J. Antiangiogenesis in cancer therapy: endostatin and its mechanisms of action. Exp Cell Res. 2006;312(5):594–607.
35. Xue L, Chen H, Zhang T, Chen J, Geng Z, Zhao Y. Changes in serum vascular endothelial growth factor and endostatin concentrations associated with circulating endothelial progenitor cells after acute ischemic stroke. Metab Brain Dis. 2017;32(2):641–8.

36. Kawamoto A, Katayama M, Handa N, Kinoshita M, Takano H, Horii M, Sadamoto K, Yokoyama A, Yamanaka T, Onodera R, Kuroda A, Baba R, Kaneko Y, Tsukie T, Kurimoto Y, Okada Y, Kihara Y, Morioka S, Fukushima M, Asahara T. Intramuscular transplantation of G-CSF-mobilized CD34(+)cells in patients with critical limb ischemia: A phase I/IIa, multicenter, single-blinded, dose-escalation clinical trial. Stem Cells. 2009;27(11):2857–64.

37. Dong Z, Chen B, Fu W, Wang Y, Guo D, Wei Z, Xu X, Mendelsohn FO. Transplantation of purified CD34+ cells in the treatment of critical limb ischemia. J Vasc Surg. 2013;58(2):404–411 e403.

38. Madaric J, Klepanec A, Valachovicova M, Mistrik M, Bucova M, Olejarova I, Neepal R, Madaricova T, Paulis L, Vulev I. Characteristics of responders to autologous bone marrow cell therapy for no-option critical limb ischemia. Stem Cell Res Ther. 2016;7(1):116.

39. Qin Q, Qian J, Ma J, Ge L, Ge J. Relationship between thrombospondin-1, endostatin, angiopoietin-2, and coronary collateral development in patients with chronic total occlusion. Medicine (Baltimore). 2016;95(33): e4524.

40. Golledge J, Clancy P, Hankey GJ, Yeap BB, Norman PE. Serum endostatin concentrations are higher in men with symptoms of intermittent claudication. Dis Markers. 2014:298239.

41. Ziegelhoeffer T, Fernandez B, Kostin S, Heil M, Voswinckel R, Helisch A, Schaper W. Bone marrow-derived cells do not incorporate into the adult growing vasculature. Circ Res. 2004;94(2):230–8.