Advances in Medical Sciences 63 (2018) 140–146

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original research article

The application of L-PRP in AIDS patients with crural chronic ulcers: A pilot study

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ARTICLE INFO

Article history:
Received 14 February 2017
Accepted 17 October 2017
Available online 6 November 2017

Keywords:
Healing
Growth factors
Platelet-rich plasma
Ulcers
Stem cells

ABSTRACT

Purpose: Nonhealing wounds or skin ulcerations are the result of insufficient repair and destruction of a local healing potential. Opportunistic infections which cause a lot of ulcer complications influence the worsening general condition of patients with AIDS, ultimately leading to death. The chronicity of the condition and poor results of conventional therapy have prompted the search for new methods of treatment.
Materials and methods: We have examined venous or arteriovenous insufficiency-related extensive crural ulcers in AIDS patients. Crural ulcer healing processes were evaluated with clinical observations and histopathological, immunohistochemical and molecular examinations of tissue samples harvested from the wound edges before and on day 10 after L-PRP cover dressing.
Results: Clinical observations showed positive effects of L-PRP in all patients. However, complete wound closure was noted in 60% of cases. Statistical analysis of histological examination showed increased epidermal processes between samples, but the difference was nonsignificant. However, immunohistochemical investigations showed an increased healing process with strong statistical significance. The mean VEGF level before L-PRP usage was 114.3 vessels/mm^2 and on day 10 118.9 ($p = 0.001523$). The mean FLK level was 103.2 and 109.9 respectively ($p = 0.008241$). The biggest differences were observed for CD34, with values of 68.2 on day 0 and 100.8 on day 10 ($p = 0.006982$). Molecular analysis generally showed decreased gene expression and confirmed vascular formation and reepithelialization processes.
Conclusions: In our opinion, L-PRP may be used to eradicate microorganisms from wounds, to induce neovascularization, and in unhealed cases prepare the base and edge of the ulcer for skin grafting and tissue expansion procedures.

1. Introduction

The number of HIV and AIDS patients is constantly growing. Severe symptoms of HIV infection and AIDS may not appear for many years. A characteristic trait of AIDS is irreversible and cachectic course of the pathogenic process, which leads to infections caused by opportunistic microorganisms. The chronic wounds induced by venous or arteriovenous insufficiency in patients with diabetes or immunological defects such as AIDS pose a considerable problem [1]. No fully efficient therapeutic solution exists, and many wounds are nearly impossible to close [2]. Due to the lack of improvement in conventional methods of treatment, new alternatives should be sought, that would be of substantial value for the treatment of infection-related chronic ulcers, such as a gelatinous mass forming a barrier against microbes.

In recent years, the use of platelet concentrates to enhance bone regeneration and soft-tissue maturation has increased chronic healing disorders, with many attempts having been made to explain their properties of stimulating and accelerating tissue healing [2]. For years, the properties of the concentrates have been believed to be mainly related to the high concentrations of platelet-derived growth factors in their composition [2]. However, several authors have started pointing out the key role of the fibrin

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https://doi.org/10.1016/j.advms.2017.10.002
1896-1126/

clot architecture and the leukocyte content of these preparations, developing the concept that growth factors alone cannot account for all the properties in question [3,4], especially in the case of extensive soft tissue defects with coexisting infection, where ulcer healing often takes as long as weeks [5,6].

In consequence, of 4 families of platelet concentrates available, 2 families have a lower count of leukocytes: P-PRP/P-PRF (Pure-Platelet-rich Plasma/Fibrin), whilst 2 families have significant concentrations of leukocytes: L-PRP/L-PRF (Leukocyte- and Platelet-rich Plasma/Fibrin) [3].

A few publications have demonstrated that it is not only growth factors that are secreted from PRPs, but also antimicrobial peptides, resulting with a potential for stimulating healing processes in chronic ulcers [7].

The combination of inductive and antimicrobial activity of L-PRP may be critical for the treatment of infected chronic ulcers [6]. Clinical studies including bacteriological, histological, immunohistochemical, molecular and flow cytometry investigations are rare. Hence, we have examined the influence of L-PRP on infected chronic ulcers in AIDS patients.

2. Material and methods

5 consecutive patients from the Clinical Department of Infectious Diseases of the Medical University of Silesia were included in the study.

The following inclusion criteria were applied:

- venous or arteriovenous insufficiency-related crural ulcers in AIDS patients, diagnosed in clinical and Doppler examination
- ulcer area of 200–300 cm^2
- ulcer depth 0.45–0.60 cm
- no progressive evidence of a healing process in the affected site over the past 3 months
- no previous surgical treatment of the ulcer site during the last 6 months

Patient's age ranged from 39 to 45 years (mean 40.6 years). Postoperative examinations were performed by an independent physician to prevent bias during the leg assessment. Each participant was followed up on a regular outpatient basis with clinical examinations and functional evaluations.

2.1. Gelling L-PRP preparation procedure

108 mL of autologous whole blood with 12 mL anticoagulant (citrate phosphate dextrose) was drawn into a sterile tube and centrifuged for 15 min at 3200 rpm (GPS II Platelet Concentration System; Biomet, Warsaw, IN). The centrifuge spins at a maximum speed of 3200 rpm's at a maximum g-force of approximately 2200 g's. After centrifugation, the blood was separated into 3 basic components: red blood cells, L-PRP, sometimes referred to as "buffy coat," and acellular plasma (AP). Twelve milliters of L-PRP were obtained and mixed with 3 mL 1600 U/mL bovine thrombin in a 10% calcium chloride solution (Biomed, Lublin, Poland) at room temperature to form gelatinous L-PRP.

2.2. Surgical procedure

All procedures were carried out in the operating room. The bacteriological samples from the wounds were collected (day 0, 5 and 10), and semi-quantitative analysis was based on grading bacterial growth as scant, light, moderate or heavy (or 1+, 2+, 3+ or 4+), of which moderate and heavy usually indicated significant bacterial counts [8].

Patients' wounds were cleaned by the same experienced surgical team under local anaesthesia. Next, specimens of tissue sized 0.3 × 1 cm and 0.6 cm deep from wound edges were excised for histological and immunohistochemical analysis. Tissue specimens (sized 0.2 × 0.1 cm) were also surgically obtained from the ulcers for molecular investigation (Fig. 1). As the next step, an impermeable dressing (Opsite, Smith&Nephew) fixed with a glue (ConvaTec) was used, and then L-PRP was injected into the closed space between the wound and the dressing, filling it up completely.

The glued dressing was then kept for 120 h. All examinations were performed at 5, 6, 7, 8, 9 and 10 days after operation. On day 10, a second sample was excised next to the original one.

The state of the wound on day 0 as control was compared with the results obtained on day 10 after L-PRP application. For molecular investigation, a control group was created, and tissue samples were collected during 20 ankle replacement procedures. Tissue samples in dedicated liquids were promptly transported to the Morphology and Histopathology Department and Department of General Biology of the Medical University of Silesia.

2.3. Histological and immunohistochemical analysis

The sampled tissues were secured in 10% buffered formalin solution. Tissue sections were routinely processed by paraffin technique. The specimens were examined with a Labophot 2 (Nikon) multi-viewing microscope with a manual indicator at ×100 (ocular lens ×10, objective lens ×10), ×200 (ocular lens ×10, objective lens ×20) and ×400 (ocular lens ×10, objective lens ×40) magnifications. The results for each slide were noted in a histopathological assessment chart, and conclusions (diagnoses) were formulated. The presence of neutrophils, erythrocytes, eosinophiles, lymphocytes, plasmocytes, macrophages, necrosis, degeneration, fibrosis with capsule formation, giant cells, granulomas, foreign bodies, steatosis, as well as morphological cytotoxity symptoms and vascular proliferation were evaluated.

Photographic recordings (microphotographs) were made with an Olympus Provis AX 70 light microscope equipped with a DP 10 digital camera with Olympus software.

To evaluate the revascularisation processes, immunohistochemical staining with monoclonal antibodies against specific human endothelial cell markers was used: CD 34–Monoclonal Mouse Anti-Human CD 34 Class II, Clone QBEnd (Dako North America Inc., Denmark and Carpinteria, California, USA); VEGF (C-1) and FLK-1, i.e. 2 VEGF receptor (A-3) (Santa Cruz Biotechnology, Inc. California, USA).

In order to obtain an objective evaluation of the intensity of the immunochemical reaction in the newly developed vessels showing

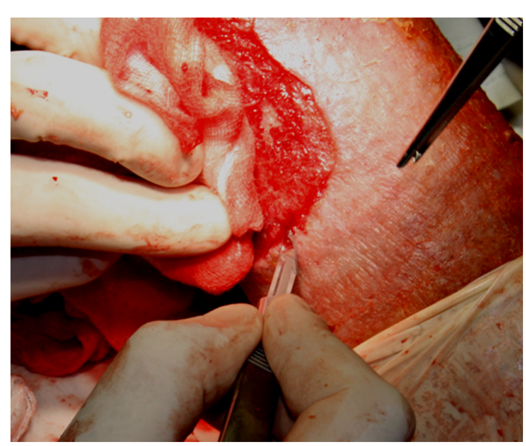

Fig. 1. The tissue specimen was excised from the wound edges.

expression for the applied antibodies (VEGF; FLK-1), a densitometric examination as well as a semi-quantitative evaluation were carried out.

For the quantitative examination (with antibodies against VEGF and FLK-1) of angiogenesis intensity in humans, the areas of interest (AOI) were chosen according to P. B. Vermeulen's standards. The following parameters were applied: 2080×1544 pixels, with microphotography objective lens $\times 10$, which corresponds to a field of vision of $718 \times 532\ \mu m$, i.e. $381976\ \mu m^2$. The obtained results were recorded in Microsoft Excel 07 sheets.

2.4. Molecular analysis

The tissue specimens after surgical excision were stored in RNAlater™ (Sigma, USA). The tissue fragments were homogenised using Lysing Matrix D (MP Biomedicals, USA) on FastPrep 24® homogeniser (MP Biomedicals, USA). Total RNA was extracted using a commercial RNeasy® Mini Kit (Qiagen, Germany). Additionally, we performed DNase I digestion using kit RNase Free DNase I Set (Qiagen, Germany) to remove trace amounts of genomic DNA.

RNA qualitative and quantitative assays were performed by Agilent 2100 Bioanalyzer (Agilents Technologies, Germany) using Agilent RNA 6000 Nono Kit (Agilents Technologies, Lithuania). cDNA synthesis was carried out using High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, USA), following the protocols provided by the manufacturer. Gene expression of VEGF-A, IL-1a, EGF, CD34, TGF-β1 was measured by quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) on the Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, USA) using TagMan®Gene Expression Assays, FAM™ dye – labeled MGB probes, (Applied Biosystems, USA) for VEGF-A – vascular endothelial growth factor A (Rn01511605_m1); IL-1a – interleukin 1, alpha (Rn00566700_m1); EGF – epidermal growth factor (Rn00563336_m1); CD34 (Rn03416140_m1) and ACTB – actin beta (endogenous control) genes. For TGF-β1– transforming growth factor, β1 was used Custom TagMan®Gene Expression Assay, cat no. 4331348, (Applied Biosystems, USA).

2.5. Cell preparation, flow-cytometric analysis, and cell sorting

Blood and L-PRP samples were processed under standardized and optimized conditions within less than 5 h after collection. For each sample, 8-fold staining with fluorochrome-conjugated mouse anti-human monoclonal antibodies was performed two times.

The antibody set was designed to allow leukocyte analysis and also the antibodies against lineage-specific progenitor cell surface markers were used: hematopoietic line (CD34+/CD45+) and peripheral blood-derived endothelial progenitor cells (CD34 +/CD45-).

After staining, erythrocyte lysis and fixation were performed with FACSLyse Solution (Becton Dickinson). Subsequently, the sample was washed with CellWash solution (Becton Dickinson), and finally resuspended in FACSFlow solution (Becton Dickinson).

Acquisition of data was performed in 8-color 3-laser flow cytometer FACSCanto II (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). The data were acquired and analyzed with Diva software (Becton Dickinson).

2.6. Statistical analysis

Statistical analysis was performed with Statistica for Windows version 8.0 (Statsoft, Kraków, Poland). Kolmogorov-Smirnov test, t-Student, Mann-Whitney *U* test and ANOVA with post-hoc RIR Tukey and Kruskal-Wallis test were used. Probability values of $p < 0.05$ were considered to be significant. The differences between the number of women and men and control and experimental groups were nonsignificant (χ^2 test).

University Ethics Committee approval was obtained for the study (Ref. No. KNW/0022/KB1/133/I/08).

3. Results

No complications related to the surgical technique were observed. 2 patients experienced moderate discomfort at their donor sample site, which generally resolved within 24 h. Mean hospitalization time per patient was 7.1 days. In all cases, the healing processes on day 10 were clinically evaluated. However, in 3 out of 5 cases, the soft tissue defect took an average of 9 weeks to heal. No skin graft operation was performed to the end of the study (12 weeks) (Table 1, Fig. 2). Two patients did not exhibit a positive final outcome. In the first case, the patient struck her cruris against a hard object, and in the remaining case the ulcer failed to heal for unknown reasons.

In most chronic wounds, Staphylococcus aureus and Pseudomonas aeruginosa were detected on day 0. On the next bacteriological examination on day 5 and 10, the detected bacterial colonization level was lower.

Patients had haemoglobin of 9.8 g/dL on average. The RBC count ranged from 3.02 million/mm3 to 6.1 million/mm3 (4.2 on average), and haematocrit was 41% on average. A normal platelet count between 150.000–400.000 was noted in all cases (211.500 on average). Total leukocyte count was 4053 cells/mm3 on average. The average platelet counts were increased by 510% (1.078.650 thrombocytes/mm3), and the mean leukocyte number increased by 590% on average after centrifugation (23912.7 cells/mm3) in L-PRP. The CD4/CD8 ratio in both patients was below 1, which is characteristic for AIDS. Peripheral blood-derived endothelial and hematopoietic progenitor cells were also detected in L-PRP samples in fourfold concentrate in comparison to the baseline level.

Statistical analysis of histological examination showed increased epidermal processes between sample 1 and 2, but the difference was nonsignificant (Fig. 3A and B). However, immunohistochemical investigations showed an increased healing process with strong statistical significance. The mean VEGF level before L-PRP usage was 114.3 vessels/mm^2 and on day 10 118.9 ($p = 0.001523$). The mean FLK level was 103.2 and 109.9 respectively ($p = 0.008241$). The biggest differences were observed for

Table 1
Clinical characteristics of AIDS ulcer group.

No	Sex	Age	Ulcer site	Ulcer area [cm^2]	Present infection	Healing	Time to heal [weeks]
1	F	39	Tibia	300	Y	Y	8
2	M	45	Tibia	260	Y	N	–
3	F	41	Tibia	250	Y	N	–
4	F	39	Tibia	260	Y	Y	9
5	M	39	Tibia	260	Y	Y	10

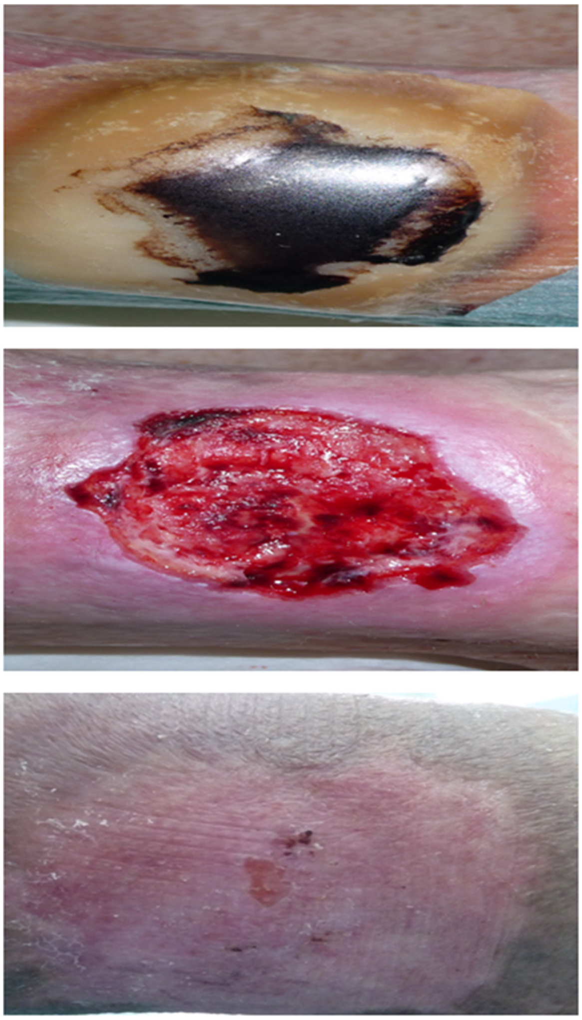

Fig. 2. Healed wound in a 39 years old woman with AIDS (Day 0, 5 and 10).

CD34, with values of 68.2 on day 0 and 100.8 on day 10 ($p = 0.006982$) (Figs. 4 –6 A and B), (Table 2).

Molecular analysis generally showed a decrease of gene expression, with VEGF gene expression decrease from 1.110000 in sample 1 to 0.612000 in sample 2 in comparison to the tissue harvested from healthy skin. The gene expression of IL-1a (18.82738 vs. 4.82300), CD34 (0,97623 vs. 0.14910), TGF beta (6.43402 vs. 1.33425) was also lower in most samples, and for IL-1a it was significant ($p = 0.008612$). The mean level of EGF beta gene expression was 0.0167 after 10 days, but in sample 1 gene expression was too low for bioanalyzer detection, and as a result statistical analysis could not be performed.

4. Discussion

Chronic nonhealing wounds or skin ulcerations are the result of insufficient repair and destruction of the local healing potential. The most common aetiology of cutaneous ulcers is decreased skin perfusion caused by arterial stenosis and additional infection [10]. Venous hypertension, extensive tissue trauma or pressure also cause this decreased skin and subcutaneous perfusion, leading to tissue ischaemia and necrosis. Traditional treatment of nonhealing wounds has consisted of a passive attempt to protect the local environment to promote the repair of tissue loss [6,10]. Antibiotic therapy reduces the bacterial count in the wound, protective dressings are used to decrease tissue trauma and enhance repair.

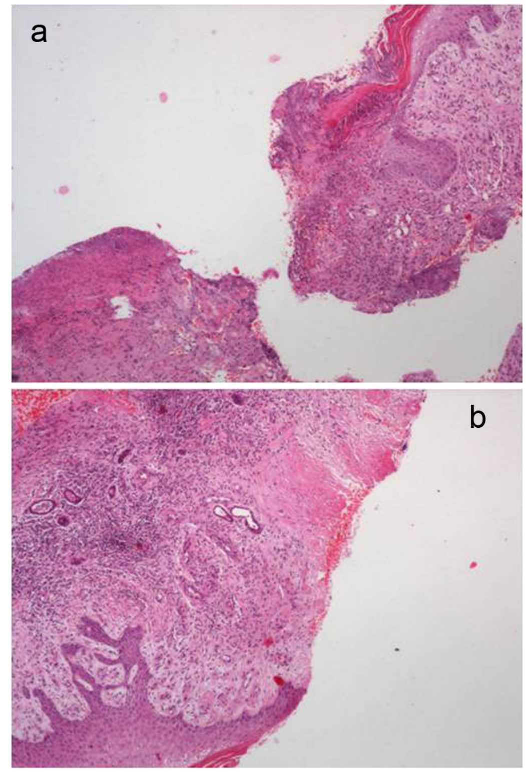

Fig. 3. Hematoxylin and eosin staining of tissue sections of 1st sample in AIDS patient (A) and 10 days after L-PRP application (B); the epidermal processes increase (Labophot 2 – Nikon, zoom ×40).

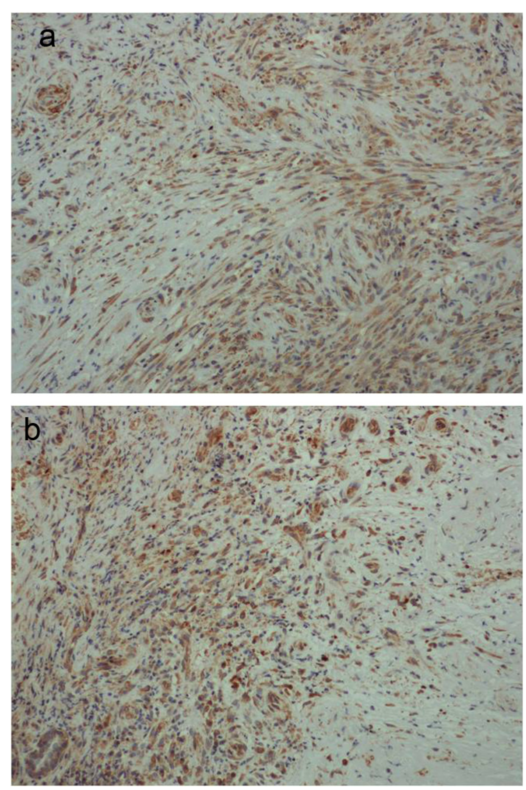

Fig. 4. VEGF immunoexpression of 1st sample in AIDS patient (A) and 10 days after L-PRP application (B); the newly developed vessels showing expression for the applied antibodies (Labophot 2 – Nikon, zoom ×100, Monoclonal Mouse Anti-Human VEGF).

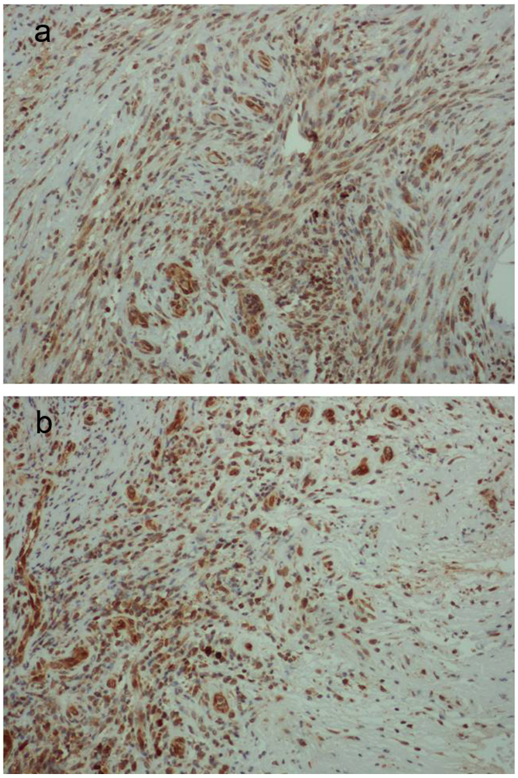

Fig. 5. FLK-1 immunoexpression of 1st sample in AIDS patient (A) and 10 days after L-PRP application (B); the newly developed vessels showing expression for the applied antibodies (Labophot 2 – Nikon, zoom ×100, Monoclonal Mouse Anti-Human FLK-1).

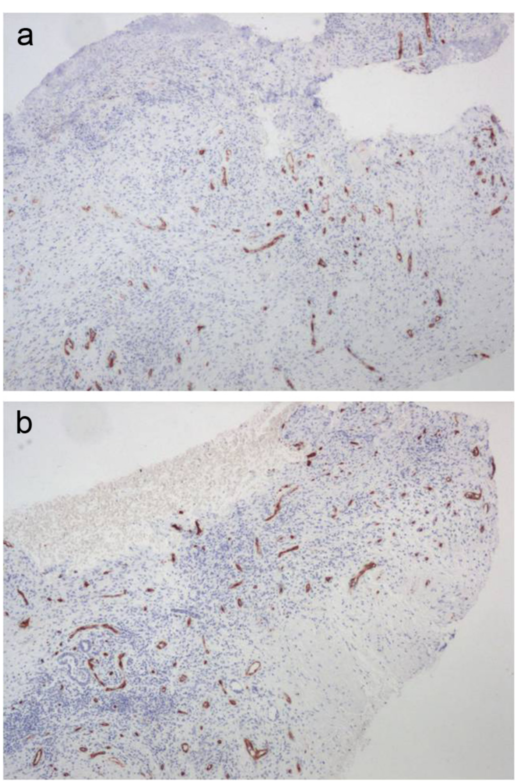

Fig. 6. CD 34 immunoexpression of 1st sample in AIDS patient (A) and 10 days after L-PRP application (B); the increase expression of CD 34 discriminates neovascularization (Labophot 2 – Nikon, zoom ×40, Monoclonal Mouse Anti-Human CD 34 Class II).

Table 2
Immunohistochemical analysis on day 0 (sample 1) and 10 (sample 2).

	Mean	SD	p
VEGFi(1)	114,3041	1,511030	
VEGFi(2)	118,9011	1,760583	0.001523
FLKi(1)	103.2	3,489035	
FLKi(2)	109.9	4,283450	0.008241
CD34i(1)	68.2	11.92391	
CD34i(2)	100.8	6,034900	0.006982

Also, topical agents are utilized, and debridement and removal of wound exudates are attempted [10,11].

In patients with high immunosuppression, e.g. AIDS, microorganisms which exist in a neutral form in healthy people become pathogenic, particulary Candida albicans, Herpes simplex, or Herpes zoster [12]. Pneumocystis carinii, Cryptococcus neoformans, Toxoplasma gondii, CMV and EBV are other microorganisms which are frequently found in HIV-positive patients. Opportunistic infections which cause a lot of ulcer complications influence the worsening general condition of HIV-positive patients, ultimately leading to death [12]. The condition's chronicity and poor results of conventional therapy have prompted the search for new, minimally invasive methods of treatment.

The use of leukocyte and platelet concentrates enrich in various cells technologies for the collection and use of natural antimicrobial agents of the microorganism is a very interesting approach, but the topic has hardly been investigated in the literature so far [13].

In these investigations, López at al. noted MRSA and MSSA growth at 6 h to be significantly inhibited by all blood concentrates, with the exception of heat-inactivated plasma (IP). MSSA was more sensitive to treatments than MRSA. Moreover, at 24 h, it was observed that leukocyte-poor gel, known as gelling P-PRP, showed a significant inhibition of MRSA growth when compared to IP. In our study, in most chronic wounds Staphylococcus aureus and Pseudomonas aeruginosa were detected on day 0, whereas on the next bacteriological examination on days 5 and 10, bacteria were detected in lower concentration.

Bielecki and Yuan performed studies on the composition and application of L-PRP. They have demonstrated these blood components to show a high concentration of leukocytes and platelets rich in natural antimicrobial peptides, which can be used for therapeutic tissue regeneration in patients with nonhealing wounds [14–16]. In our study, despite the presence of a complicating infection and chronicity, soft tissue healing processes were enhanced after the application of gelling L-PRP.

Similarly to our study, Crovetti et al. used platelet-rich plasma in 24 patients with single or multiple cutaneous ulcers with different ethiopathogenesis, such as diabetes, venous insufficiency, infection and post-traumatic condition, neuropathy or vasculitis [17]. The median initial wound size was 67.4 cm^2 (range 0.5–560 cm^2), with a median depth of 0.69 cm (range 0.2–3 cm). Ulcer duration prior to L-PRP use ranged from one month to 30 years. The authors formed their conclusions based on clinical observation. After 10-fold L-PRP application, complete wound closure was reached in 9 patients, 2 underwent a cutaneous graft, 4 discontinued treatment, and 9 had partial response and were continuing treatment. After the first use, however, granulation tissue formation increased in all cases, even though complete re-epithelialization required different time due to the varying size and duration of the ulcers. The authors noted pain relief in all patients. They concluded that topical hemotherapy with L-PRP may be considered as useful adjuvant treatment for cutaneous ulcers in a multidisciplinary management process. However, in our study, we applied L-PRP in patients with AIDS and arteriovenous leg ulcers. Unlike postoperative wounds, where percutaneous injection could performed and the whole content of L-PRP could be located in a closed area, thus keeping the substances continuously active, external application on a skin defect might only be effective for a limited time.

Moreover, many researchers use L-PRP spray application [18–20], and a dressing is used which may absorb the serum enriched with the active substances from L-PRP, and reduce the time of L-PRP activity. For this reason, we suggest the use of an impermeable dressing fixed with a special glue first, and then filling up the closed space between the wound and the dressing with L-PRP. The glued dressing is then kept on for 120 h. In our previous studies, we used a resorbable membrane as the dressing. However, the strength of the membrane decreased with time, and after a few days L-PRP leakage occurred [6].

Driver et al. carried out the first reported prospective, randomized, controlled multicenter trial regarding the use of L-PRP for the treatment of diabetic foot ulcers [21]. In that study, 129 patients were screened. 72 completed a 7-day screening period and met the study inclusion criteria. Patients were randomized into two groups: a group who received standard care with platelet-rich plasma gel, and a control (saline gel) dressing group. Wounds were evaluated biweekly for 12 weeks or until healing. Healing was confirmed 1 week following closure, and monitored for another 11 weeks. An independent audit led to the exclusion of 32 patients from the final analysis because of protocol violations and failure to complete treatment. The authors noted that 68.4% (13/19) of cases in L-PRP group and 42.9% (9/21) in the control group healed. The authors have suggested that the majority of nonhealing diabetic foot ulcers treated with autologous platelet-rich plasma gel can be expected to heal. In our study, 3 out of 5 cases healed completely. The worse efficiency may be caused by the high immunodeficiency and advanced vessel failure [22–24]. Most likely, for such cases simultaneous application of L-PRP and autologous skin grafts would be required.

Some authors reported that PRPs might not produce the desired stimulatory response when autologous graft enrich in progenitor cells is not present in the defect, because vital cells are needed for this stimulation to occur [14]. Raposio et al. added adipose-derived stem cells (ASCs) to platelet-rich plasma solution to obtain an enhanced PRP (e-PRP). They observed in e-PRP group faster healing processes in comparison with the control group [25].

The wound healing processes of these lesions were described and analyzed using noninvasive procedures, e.g. sample fluid harvesting from the skin lesion. Several studies have demonstrated that the extravasated fluid from a venous leg ulcer has increased levels of cytokines like TNFα, IL-1, IL-6 [26,27], and low concentrations of growth factors [27]. The local regulation of the mediators has a considerable influence on the final results of chronic wounds, and the plasma constituents released following blood vessel disruption are of great importance for the stimulation of wound healing [10]. A logical assumption is that the application of concentrates rich in thrombocytes, leukocytes and various substances in these wounds can only have beneficial effects on such lesions. In our study, the ulcer healing processes were evaluated not only with clinical observations. Improvement of wound vascularity was confirmed by immunohistochemistry, showing markedly increased staining for VEGF and FLK-1, L-PRP treated patients. These results suggest that the expression of the VEGF and its receptor (FLK-1) might be associated with vessel proliferation in the ulcer edge. We also conducted detailed examinations of vessel-forming processes in wound tissues by staining for CD34, which is specific for cells that are 3,3′-diaminobenzidine-positive and morphologically consistent with endothelial cells (a marker for vessel formation), with analysis showing increased CD34 expression [28,29].

For molecular investigation, a control group was created, and tissue samples were collected during 20 ankle-replacement procedures. The tissue samples were also collected prior to and on day 10 after L-PRP application, and gene expression for healing process markers was evaluated. Hypoxia upregulates tissue expression of VEGF and CD34 and its receptors, which in turn promotes an angiogenic response. Hypoxia, through hypoxia inducible factor (HIF)-1alpha, strongly induces the expression of VEGF. A gradient of VEGF expression is established that parallels the hypoxic gradient, and e.g. endothelial cells subsequently migrate towards the most hypoxic areas, secreting VEGF [30]. During healing processes and vascular formation hypoxia decreases and expression of VEGF and CD34, is reduced. In our study, VEGF and CD34 gene expression was decreased after 10 days vs. day 0. Moreover, IL-1 gene expression was significantly reduced at the wound site and reepithelialization occurred. Nevertheless, on day 0, the level of EGF beta gene expression in the samples was too low for bioanalyzer detection, and statistical analysis could not be performed [31].

This is a pilot study on the use of L-PRP as biologic treatment for chronic ulcers, and has been limited to 5 cases only. The positive effects of L-PRP were obvious in all cases, and a complete wound closure was noted in 3 out of 5 patients (60%). To our knowledge, this is the first study investigating the effect of L-PRP delivery to a nonhealing site in AIDS patients. We believe that percutaneous autologous L-PRP grafting can be an effective and safe method for the treatment of chronic wounds. L-PRP may be used to clean them from microorganisms, to induce neovascularization and in unhealed cases to prepare the base and edge of the ulcer for skin grafting and tissue expansion procedures. However, further studies are needed to validate this experimental approach.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by the Committee for Scientific Research, Polish Ministry of Science, No. N403 070 32/4120. We would like to thank doctors and laboratory workers from Central Laboratory PSK I, Zabrze, Poland; Department of Histology, Katowice, Poland; Department of Molecular Biology, Zabrze, Poland and Clinic of Infectious Diseases, Chorzów, Poland for technical help.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

[1] Diz Dios P, Scully C. Antiretroviral therapy: effects on orofacial health and health care. Oral Dis 2014;20(March (2)):136–45.
[2] Bielecki T, Dohan Ehrenfest DM. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF): surgical adjuvants, preparations for in situ regenerative medicine and tools for tissue engineering. Curr Pharm Biotechnol 2012;13(June (7)):1121–30.
[3] Dohan Ehrenfest DM, Bielecki T, Mishra A, Borzini P, Inchingolo F, Sammartino G, et al. In search of a consensus terminology in the field of platelet concentrates for surgical use: platelet-rich plasma (PRP), platelet-rich fibrin (PRF), fibrin gel polymerization and leukocytes. Curr Pharm Biotechnol 2012;13(June (7)):1131–7.
[4] Clark RA. Fibrin and wound healing. Ann N Y Acad Sci 2001;936:355–67.
[5] Gibble JW, Ness PM. Fibrin glue: the perfect operative sealant? Transfusion 1990;30:741–7.
[6] Cieslik-Bielecka A, Choukroun J, Odin G, Dohan Ehrenfest DM. L-PRP/L-PRF in esthetic plastic surgery, regenerative medicine of the skin and chronic wounds. Curr Pharm Biotechnol 2012;13:1266–77.

[7] Cieslik-Bielecka A, Dohan Ehrenfest DM, Lubkowska A, Bielecki T. Microbicidal properties of leukocyte- and platelet-rich plasma/fibrin (L-PRP/L-PRF): new perspectives. J Biol Regul Homeost Agents 2012;26:43S–52S.
[8] Bowler P, Duerden B, Armstrong D. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 2001;14:244–69.
[10] Cieslik-Bielecka A, Bielecki T, Gazdzik TS, Arendt J, Krol W, Szczepanski T. Autologous platelets and leukocytes can improve healing of infected high-energy soft tissue injury. Transfus Apher Sci 2009;41(1):9–12.
[11] Scott MG, Hancock RE. Cationic antimicrobial peptides and their multifunctional role in the immune system. Crit Rev Immunol 2000;20:407–31.
[12] Rasero L, Simonetti M, Falciani F, Fabbri C, Collini F, Dal Molin A. Pressure ulcers in older adults: a prevalence study. Adv Skin Wound Care 2015;28:461–4.
[13] López C, Alvarez ME, Carmona JU. Temporal bacteriostatic effect and growth factor loss in equine platelet components and plasma cultured with methicillin-sensitive and methicillin-resistant staphylococcus aureus: a comparative in vitro study. Vet Med Int 2014;2014:525826.
[14] Bielecki T, Gazdzik TS, Szczepanski T. Benefit of percutaneous injection of autologous platelet-leukocyte-rich gel in patients with delayed union and nonunion. Eur Surg Res 2008;40:289–96.
[15] Wang HF, Gao YS, Yuan T, Yu XW, Zhang CQ. Chronic calcaneal osteomyelitis associated with soft-tissue defect could be successfully treated with platelet-rich plasma: a case report. Int Wound J 2013;10:105–9.
[16] Liu J, Yuan T, Zhang C. Three cases using platelet-rich plasma to cure chronic soft tissue lesions. Transfus Apher Sci 2011;45:151–5.
[17] Crovetti G, Martinelli G, Issi M, Barone M, Guizzardi M, Campanati B, et al. Platelet gel for healing cutaneous chronic wounds. Transfus Apher Sci 2004;30(2):145–51.
[18] Ghassab S, Dulin J, Bertone AL. Thromboelastographic clot characteristics of autologous equine blood products after activation by autologous thrombin, bovine thrombin, or calcium chloride. Vet Surg 2015;44:970–5.
[19] James R, Chetry R, Subramanian V, Ashtekar A, Srikruthi N, Ramachandran S, et al. Platelet-rich plasma growth factor concentrated spray (Keratogrow®) as a potential treatment for androgenic alopecia. J Stem Cells 2016;11:183–9.
[20] Anandan V, Jameela WA, Saraswathy P, Sarankumar S. Platelet rich plasma: efficacy in treating trophic ulcers in leprosy. J Clin Diagn Res 2016;10:WC06–9.
[21] Driver VR, Hanft J, Fylling CP, Beriou JM. Autologel diabetic foot ulcer study group.: a prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers. Ostomy Wound Manage 2006;52(6)68–70 72,74.
[22] Reis Machado J, da Silva MV, Cavellani CL, dos Reis MA, Monteiro ML, Teixeira Vde P, et al. Mucosal immunity in the female genital tract, HIV/AIDS. Biomed Res Int 2014;2014:350195.
[23] Camoni L, Regine V, Stanecki K, Salfa MC, Raimondo M, Suligoi B. Estimates of the number of people living with HIV in Italy. Biomed Res Int 2014;2014:209619.
[24] Cieslik-Bielecka A, Sędek L, Beniowski M, Gazdzik TS, Bielecki T, Ostałowska A, et al. Analysis of B, NK, T lymphocytes for CD4, CD8, CD25, CD69 and HLA-DR in blood and platelet-rich plasma in AIDS patients with oral ulcers ? preliminary report. Pol J Environ Stud 2008;17:166–70.
[25] Raposio E, Bertozzi N, Bonomini S, Bernuzzi G, Formentini A, Grignaffini E, et al. Adipose-derived stem cells added to platelet-rich plasma for chronic skin ulcer therapy. Wounds 2016;28:126–31.
[26] Beidler SK, Douillet CD, Berndt DF, Keagy BA, Rich PB, Marston WA. Inflammatory cytokine levels in chronic venous insufficiency ulcer tissue before and after compression therapy. J Vasc Surg 2009;49:1013–20.
[27] Murphy MA, Joyce WP, Condron C, Bouchier-Hayes DA. reduction in serum cytokine levels parallels healing of venous ulcers in patients undergoing compression therapy. Eur J Vasc Endovasc Surg 2002;23:349–52.
[28] Syeda MM, Jing X, Mirza RH, Yu H, Sellers RS, Chi Y. Prostaglandin transporter modulates wound healing in diabetes by regulating prostaglandin-induced angiogenesis. Am J Pathol 2012;181:334–46.
[29] Menon P, Fisher EA. Immunostaining of macrophages, endothelial cells, and smooth muscle cells in the atherosclerotic mouse aorta. Methods Mol Biol 2015;1339:131–48.
[30] Molitoris KH, Kazi AA, Koos RD. Inhibition of oxygen-induced hypoxia-inducible factor-1alpha degradation unmasks estradiol induction of vascular endothelial growth factor expression in ECC-1 cancer cells in vitro. Endocrinology 2009;150:5405–14.
[31] Lin ZQ, Kondo T, Ishida Y, Takayasu T, Mukaida N. Essential involvement of IL-6 in the skin wound-healing process as evidenced by delayed wound healing in IL-6-deficient mice. J Leukoc Biol 2003;73:713–21.