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The association between early postoperative healing and the 12-month clinical and radiographic outcomes of guided tissue regeneration in aggressive periodontitis patients

Wpływ wczesnego gojenia pozabiegowego na 12-miesięczne kliniczne i radiologiczne wyniki sterowanej regeneracji tkanek u pacjentów z agresywnym zapaleniem przyzębia

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Summary

Aim: This study evaluates the influence of early healing on clinical and radiological outcomes of guided tissue regeneration (GTR) procedures of vertical intrabony defects in patients with aggressive periodontitis (AgP) in a 12-month follow-up. The influence of patient-related, site-specific and technical aspects on optimal early wound healing was also assessed.

Material/Methods: This analysis included 25 patients with 61 intrabony defects. All sites were treated according to guidelines of minimally invasive surgical technique with the use of bone grafts and collagen membranes. Early post-operative healing was evaluated using the Early Wound-Healing Index (EHI). Changes in clinical and radiological parameters were assessed 12 months postoperatively.

Results: After 2 weeks, primary healing (EHI ≤3) was observed in 44 sites (72.13%) and secondary healing was present in 17 sites (22.87%) (EHI = 4). The presence of thin gingival phenotype was significantly associated with an increased risk of secondary healing (OR = 0.203; p = 0.014). At 12 months, GTR resulted in a significant clinical attachment level gain, as well as probing pocket depth reduction and radiographic defect depth reduction. Primary or secondary healing did not affect these outcomes.

Conclusions: Thick gingival biotype might be a prerequisite for optimal early wound healing. However, the type of early healing seems not to affect the long-term outcomes after regenerative treatment in aggressive periodontitis patients.

Keywords: **Aggressive periodontitis • Guided tissue regeneration • Reparation • Wound healing**

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INTRODUCTION

Wound healing is a process that has been thoroughly studied and described by many authors [18]. The healing processes can be affected through regeneration, where all lost tissues are restored along with their function, or through reparation leading to the formation of tissue scarring [32].

Interruption of tissue continuity during surgery leads to damage of blood vessels and bleeding. The contact of blood with the tissue factor initiates the extrinsic coagulation cascade, whereas contact with collagen activates the intrinsic pathway. These processes lead to the activation of thrombocytes and the polymerization of fibrin, resulting in clot formation [33]. The clot stops bleeding, covers damaged tissues and constitutes a matrix for migrating cells: leukocytes, keratinocytes, fibroblasts and endothelial cells [32]. In the first inflammatory phase, which takes place immediately after the injury, tissue edema and migration of neutrophils and monocytes occur as a result of vasodilatation and increased permeability of blood vessel walls. After 3 days, macrophages appear at the site of injury, which triggers the phagocytosis of pathogens and products resulting from the breakdown of cells [37]. Macrophages are responsible for synthesizing numerous growth factors involved in wound healing (TGF-β, TGF-α, FGF, PDGF and VEGF) by stimulating cell proliferation and synthesis of extracellular matrix [9]. The proliferation phase, lasting 3 to 21 days after the injury, involves the formation of cell-rich granulation tissue followed by its maturation and remodeling. During these processes, fibroblasts migrate into the fibrin matrix, which is accompanied by neovascularization, angiogenesis and epithelialization of wound edges [4]. During the maturation of the granulation tissue, fibroblasts produce extracellular matrix rich in collagen. The collagen matrix production process is completed by the transformation of fibroblasts into actin-producing myofibroblasts, which is responsible for contracting wound margins [30]. The last phase of remodeling can take up to 12 months from the moment of the injury. Reconstruction of the extracellular matrix primarily involves the production of type I collagen, which replaces type III collagen formed in the proliferation phase. During maturation, tissues may be repaired or regenerated, which depends on the migration sequence and the maturation of respective cells [15].

The biological and molecular basis of the underlying wound healing mechanisms is also applicable to the processes occurring in the oral cavity after periodontal surgery procedures.

Aggressive periodontitis (AgP) is characterized by a rapid loss of clinical attachment and alveolar bone, a disproportion between the number of dental plaque deposits and the severity of the disease, the absence of general diseases and frequent occurrence within a family [21]. AgP constitutes a significant health problem as it may lead to tooth loss, masticatory dysfunction, disability and subsequently may impair the quality of life. It is a complex disease whose occurrence and course depend on both genetic and environmental factors [3]. The incidence of AgP in the population is estimated at 0.5–2.5% [34]. It should be mentioned though that the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions stated that there is no evidence to consider AgP as a pathophysiologically distinct disease; hence, case definitions of periodontitis should be based on a blend of periodontitis stage and periodontits grade [36]. As this study included cases treated long before the new classification, we followed the system introduced by the 1999 International Workshop on Classification of Periodontal Diseases that differentiated between AgP and chronic periodontitis (ChP) [2].

Guided tissue regeneration (GTR) aims at restoring all structures lost in the course of periodontitis, i.e. alveolar bone, root cement and collagen fibers of the periodontal ligament. At the core of tissue regeneration are complex processes and interactions at the molecular and cellular level. A prerequisite for regeneration of periodontal tissues is to obtain clot adhesion to the root surface of the tooth and to ensure its proper stabilization during healing [35]. Dehiscence of the wound within the first weeks of surgical treatment may disturb the cascade of reactions conditioning the course of regenerative processes. In addition, in the case of use of biomaterials and barrier membranes, impaired healing can lead to partial or total loss of the graft and even to infection of the barrier membrane. These factors can impair healing and reduce the regenerative potential by promoting reparation [6].

The aim of the study was to analyze the effect of early post-surgical healing on the 12-month clinical and radiological results of guided tissue regeneration of vertical intrabony defects in patients with aggressive periodontitis. The influence of patient-related, site-specific and technical aspects on optimal early wound healing was also assessed.

MATERIAL AND METHODS

This study is a secondary analysis of data collected from three randomized clinical trials conducted at the Department of Periodontology and Oral Diseases of Medical University of Warsaw, Poland, which evaluated different regenerative strategies in patients with AgP. All of the abovementioned studies received the positive approval by the institutional review board (KB/135/2014;

KB/37/2016; KB/209/2017). All clinical procedures were carried out in accordance with the Helsinki Declaration of 1975, as revised in Tokyo in 2004. Written informed consent forms were signed by every patient.

INCLUSION CRITERIA

The study adhered to the following inclusion criteria: 1) diagnosis of aggressive periodontitis in line with definition by American Academy of Periodontology [21]; 2) no systemic diseases; 3) no consumption of medications affecting periodontal status; 4) no pregnancy or lactation; 5) no cigarette smoking; 6) history of periodontitis in parents or siblings; 7) presence of at least one tooth with probing pocket depth (PPD) ≥6 mm, clinical attachment level (CAL) ≥5 mm and intrabony defect ≥3 mm as detected in periapical radiographs; 8) full-mouth plaque index (FMPI) ≤20%; 9) bleeding on probing index (BoP) ≤20%; 10) the tooth had to be vital or properly treated; 11) no furcation involvement; 12) the width of keratinized tissue on the labial/buccal site of the tooth ≥2 mm.

PRESURGERY PROCEDURES

For each patient, a full mouth disinfection protocol (FMD) was implemented, which involved simultaneous non-surgical treatment of all the pockets during one visit (scaling and root debridement) using hand and ultrasonic instruments. Mechanical debridement was combined with the use of antiseptics in the form of rinsing liquid containing 0.2% chlorhexidine (Curasept ADS 220; Curaden AG, Kriens, Switzerland) and gel with 1% chlorhexidine (Curasept ADS 100, Curaden AG, Kriens, Switzerland). In addition, all patients received antibiotics (500 mg of amoxicillin + 250 mg of metronidazole three times per day for one week). Each patient was given individual instruction on maintaining optimal oral hygiene.

CLINICAL AND RADIOGRAPHIC ASSESSMENTS

After 6-9 weeks from non-surgical treatment, the clinical parameters were carefully evaluated by one calibrated examiner (T.K) who used a graded periodontal probe (UNC probe 15 mm; Hu-Friedy, Chicago, USA). The assessment included: 1) dichotomous (yes/no) FMPI according to O'Leary et al. [28] on four tooth surfaces (i.e. distal, buccal, mesial, lingual). The index was determined by dividing the number of surfaces with plaque by the number of all examined surfaces; 2) dichotomous (yes/no) BoP index according to Ainamo and Bay [1]. Bleeding was assessed at six points for each tooth (i.e. distobuccal, buccal, mesiobuccal, distolingual, lingual, mesiolingual). The index was determined by dividing the number of bleeding points by the number of all assessed points; 3) PPD was evaluated at six points of each tooth as a distance from the gingival margin to the bottom of the pocket; 4) CAL at six points of each tooth as a distance from the cemento-enamel junction (CEJ) to the bottom of the pocket; 5) gingival recession (GR) at the buccal point of each tooth as a distance from CEJ to the gingival margin; 6) width of keratinized tissue (WKT) was assessed mid-bucally as a distance from the gingival margin to the mucogingival junction. Mucogingival junction was demarcated by coloring the mucogingival complex with iodine solution; 7) gingival phenotype was categorized as thin if gingival thickness ≤1mm, and as thick if gingival thickness >1mm. Gingival thickness was evaluated 2 mm apical to the gingival margin by perpendicularly inserting a 10-mm endodontic spreader with a silicone stopper until the alveolar bone or root surface was reached. An electronic caliper (YATO® YT-7201; Toya, Wrocław, Poland) was used to measure gingival thickness indicated on endodontic instrument with a rubber stop; 8) interdental contact point was recorded as present or absent.

Paralleling cone technique with individual film holders and phosphor plates (KaVo Scan eXam; KaVo, Biberach, Germany) using an x-ray unit operating at 70 kV, 4 mA, and 0.1-s exposure time were employed to take standardized intraoral radiographs. The radiographs were assessed using Planmeca Romexis Viewer software (Planmeca, Helsinki, Finland). Some anatomical landmarks, such as CEJ, alveolar crest (AC) and base of the defect (BD) were selected. Subsequently, two auxiliary lines were drawn, first in the axis of the tooth (AUX1), and second (AUX2) perpendicular to AUX1 from AC. The consecutive measurements were gathered: 1) radiographic defect depth (DD) as a distance from the point where AUX2 crossed the CEJ-BD line to BD; 2) radiographic defect angle between the intersection of CEJ-BD line of the tooth and the demarcation of the wall of the defect; 3) distance from CEJ to AC (ACP).

Subjects who met all of the inclusion criteria were asked to participate in surgical treatment.

SURGICAL INTERVENTION AND INTRASURGERY EVALUATION

All defects were treated according to the guidelines of minimally invasive surgery by single experienced clinician (B.G.) [7]. The procedure was started with incisions in the interdental spaces. The choice of incision depended on the width of the interdental space. A simplified papilla preservation flap (SPPF) was used in the case of narrow spaces, and a modified papilla preservation technique (MPPT) was used in the case of wide spaces. In intermolar spaces and those with difficult access, SPPF incisions were made regardless of the width of the space. These incisions extended into grooved incisions. The extent of the cut depended on the size of the defect. If necessary, vertical incisions were prepared. Subsequently, the muco-periosteal vestibular flap was prepared. Then, interdental papillae were separated from the bone base with a scalpel and the palatal flap was raised. The next step was removal of granulation tissue from intrabony defects followed by debridement and planning of root surfaces using hand and ultrasonic tools.After surgical debridement, the following evalua-

tions were made: 1) the depth of the defect as the distance from the alveolar crest to the deepest point in the defect; 2) the width of the defect as the distance from the alveolar crest to the root surface; 3) the number of the remaining walls of the defects (defects were classified as one-wall, two-wall and three-wall defects).

Intrabony defects were filled with biomaterials (Bio-Oss®, Geistlich Biomaterials, Princeton, USA/Gen-Os®, Tecnoss, Turin, Italy/allogenic bone grafts, Department of Transplantology and Cell Tissue Bank, Medical University of Warsaw, Poland) and covered with collagen membrane (Bio-Gide®; Geistlich Biomaterials). For coronally advanced flap without tension, periosteal incision was performed at its base. Interdental spaces were closed with vertical modified mattress sutures (5/0 polypropylene monofilament suture, Prolene® 5/0 16 mm 3/8, Ethicon, Somerville, USA), and vertical incisions with simple sutures. Primary flap closure was achieved in all sites.

POST-OPERATIVE CARE AND ASSESSMENTS

After the procedure, patients were administered 600 mg of ibuprofen, the same dose was repeated after 8 hours. Patients were then given post-operative recommendations that included: 1) mouth rinsing with 0.2% chlorhexidine solution (Curasept ADS 220) 3 times a day for 1 minute; 2) no brushing of the treatment area; 3) a soft, mild diet; 4) avoiding physical effort; 5) check up visit after 7 and 14 days. During the follow-up visits, wound healing in the interdental spaces was assessed and the supragingival plaque was removed from the whole dentition using a prophylaxis brush and gel containing 1% chlorhexidine (Curasept ADS 100). The sutures were removed after 2 weeks.

Post-operative healing was assessed 2 weeks after the surgery using the Early Wound-Healing Index (EHI), as classified in the consecutive grades: 1) EHI = 1: complete flap closure, no fibrin line in the interproximal area; 2) EHI = 2: complete flap closure, thin fibrin line in the interproximal area; 3) EHI = 3: complete flap closure, fibrin clot in the interproximal area; 4) EHI = 4: incomplete flap closure, partial necrosis of the interproximal tissue; 5) EHI = 5: incomplete flap closure, complete necrosis of the interproximal tissue [39]. EHI ≤3 was regarded as primary healing, while EHI ≥4 as secondary healing.

Patients were placed on a 2-week recall system for 3 months, and a 3-month recall system for one year. 12 months after the surgery, PPD, CAL, GR, and DD were assessed.

STATISTICAL ANALYSIS

For statistical analysis the measurements at the site with the greatest presurgical CAL value were used. Data were expressed as mean ± standard deviation (SD). Statistical measurements were carried out with Statistica v. 13 (StatSoft Inc., Tulsa, USA). Any p values of less than 0.05 (p <0.05) were considered statistically significant.

Descriptive analyses on early postoperative healing were based on the entire defect population (n = 61). The patient-related (age, gender, FMPI, BoP) and site-related parameters (tooth type, tooth position, PPD, CAL, GR, presence of interdental contact point, width of interdental space, WKT, phenotype, DD, RVG angle, ACP, defect configuration, defect depth and width), together with technical aspects (presence of vertical incision, papilla preservation technique) were regarded as independent variables. Analyses were carried out for sites being categorized to EHI, which was treated as binomial variable. Analyses were performed for sites that were divided as EHI≤2 or EHI≥3, as well as comparing sites with primary healing (EHI ≤3) with sites that presented secondary healing (EHI ≥4) to evaluate which variables were associated with optimal wound healing. Comparisons between the

Table 1. Baseline clinical features for the study group

Variables	Sites (n = 59)
Tooth type (n)	
Molars	34
Premolars	15
Upper incisors, canines	12
Tooth position (n)	
Maxillary teeth	20
Mandibular teeth	41
Clinical measurements	
FMPI (%)	10.50 [8.73;12.27]±6.92
BoP (%)	13.22 [11.82;14.61]±5.45
PPD (mm)	7.28 [6.96;7.59]±1.23
CAL (mm)	8.46 [8.08;8.83]±1.47
GR (mm)	1.26 [1.02;1.50]±0.93

Variables	Sites (n = 59)
Radiographic measurements DD (mm)	5.03 [4.63;5.43]±1.56
ACP (mm)	3.92 [3.50;4.34]±1.65
RVG angle (degrees)	28.64 [26.45;30.83]±8.56
Sites-specific characteristics Interdental contact point (present/absent)	10/51
Interdental space width (mm)	2.78 [2.56;3.00]±0.87
GR (mm)	2.57 [2.38;2.76]±0.74
Phenotype (thin/thick)	14/47
Technical aspects Vertical incision (yes/no) Papilla preparation MPPT (n) SPPF (n)	 36/25 25 36
Intrabony defect characteristics	
Depth (mm)	5.00 [4.56;5.44]±1.70
Width (mm)	2.96 [2.68;3.24]±1.09
Defect configuration (n) One-wall Two-wall Three-wall	 12 20 29
Healing after 2 weeks Primary/Secondary (n) EHI 1 (n) 2 (n) 3 (n) 4 (n) 5 (n)	 44/17 35 5 4 17 0

The means with 95% CI [in brackets] and ± SD of probing values and radiographic measurements of the defects before surgery; *FMPI* – full-mouth plaque index; *BoP* – bleeding on probing index; *PPD* – probing pocket depth; *CAL* – clinical attachment level; *GR* – gingival recession; *DD* – radiographic defect depth; *ACP* – alveolar crest position; RVG – angle- radiographic defect angle; *WKT* – width of keratinized tissue; *MPPT* – modified papilla preservation technique; *SPPF* – simplified papilla preservation flap; *EHI* – Early Wound-Healing Index; *n* – number of defects; *SD* – standard deviation; *CI* – confidence interval

abovementioned variables for different levels of EHI were evaluated based on means (for quantitative variables) or percentages (for categorical variables). Comparisons of means were performed using the Student's t test for dependent samples and the Student's t test for independent observations because most of variables follow normal distribution (evaluation based on histograms and K-S test). Comparisons for categorical variables were conducted using chi-squared test. Twelve-month changes in PPD, CAL, GR and DD were determined and referred to EHI.

RESULTS

Twenty-five patients with diagnosed AgP (18 females, mean age 37.6±11.5 years and 7 males, mean age 44.7±10.8) were included in the study. However, it should be underlined that, taking into consideration the new classification system of periodontal diseases, all of the cases involved in this study would be categorized as periodontitis Stage III Grade C. Patients presented 61 intraosseous defects and 50 surgical procedures were performed. Three patients contributed 1 defect, fourteen patients contributed 2 defects, five

patients contributed 3 defects and 3 patients contributed 5 defects. In the majority of patients xenogenic grafts were used (Bio-Oss® in 43 sites, 16 subjects; and Gen-Os® in 11 sites, 5 subjects), while in 7 sites intrabony defects were filled with allogenic bone grafts (4 subjects). All patients complied with the recall program until the 12-month visit. Defect characteristics are presented in Table 1.

After two weeks EHI was 1.29 ± 1.33 and ranged from 1.0 to 4.0. Forty-four sites presented primary healing (EHI: 1–3) and seventeen sites showed incomplete flap closure and secondary healing (EHI = 4). None of the defects presented EHI = 5.

Comparisons of means or percentages for categorical variables between different levels of EHI are presented in Table 2. The only significant difference was found for phenotype. When this variable associated with early wound healing was put into a multiple regression analysis, the model was statistically significant (p = 0.014). Generally speaking, the likelihood of better healing was higher for sites with a thick phenotype (OR = 0.203) (Table 3).

GTR resulted in significant CAL gain, as well as PPD reduction and DD reduction 12 months postoperatively (Table 4). No statistically significant differences in the changes of CAL, PPD, GR, and DD were noted between either patients with EHI ≤2 and EHI ≥3 or EHI ≤3 (primary healing) and EHI = 4 (secondary healing) (Table 5).

DISCUSSION

Tissue wounds, including surgical sites, can heal by first intention (primary healing) or by granulation (secondary healing). Primary healing directly connects tissue edges the with the minimal creation of new tissue between them. During primary healing of the bone throughout the remodeling phase, an immature fibrous bone is formed, which over time is replaced by lamellar bone [25]. With respect to secondary healing, which

Table 2. Means or percentage fractions of variables for different levels of EHI and their comparisons based on t-test or chi-squared test.

Variables	EHI≤2	EHI≥3	p 1	EHI≤3	EHI=4	p 2
Age (years)	37.23	41.30	0.185	38.59	38.56	0.993
Gender (% females)	77.5%	76.2%	0.908	75.0%	82.4%	0.540
Tooth position (% mandibular teeth)	72.5%	61.9%	0.396	68.2%	70.6%	0.856
Tooth type (% molars)	60.0%	47.6%	0.230	61.4%	41.2%	0.155
FMPI (%)	9.67	12.08	0.200	9.93	11.98	0.304
BoP (%)	12.61	14.38	0.232	12.92	13.99	0.494
PPD (mm)	7.25	7.33	0.803	7.23	7.41	0.603
CAL (mm)	8.43	8.52	0.805	8.45	8.47	0.970
GR (mm)	1.25	1.29	0.888	1.30	1.18	0.658
DD (mm(	5.19	4.72	0.267	5.19	4.62	0.200
RVG angle (°)	27.69	30.44	0.237	27.69	31.08	0.167
ACP (mm)	4.06	3.67	0.388	3.98	3.77	0.662
Vertical incision (present/absent)	60.0%	57.1%	0.829	59.1%	58.8%	0.985
Defect morphology (% 3)	50.0%	42.9%	0.596	47.7%	47.1%	0.963
Defect depth (mm)	5.13	4.76	0.433	5.16	4.59	0.244
Defect width (mm)	3.03	2.83	0.518	2.98	2.91	0.835
Papilla preservation technique (% SPPF)	52.5%	71.4%	0.153	54.5%	70.6%	0.253
Interdental contact point (% present)	90.0%	76.2%	0.149	86.4%	82.4%	0.692
Interdental space width (mm)	2.80	2.74	0.795	2.84	2.62	0.375
WKT (mm)	2.58	2.57	0.986	2.64	2.41	0.292
Phenotype (% thick)	85.0%	61.9%	0.042*	86.4%	52.9%	0.005*

FMPI – full-mouth plaque index; *BoP* – bleeding on probing index; *PPD* – probing pocket depth; *CAL* – clinical attachment level; *GR* – gingival recession; *DD* – radiographic defect depth; RVG angle – radiographic defect angle; *ACP* – alveolar crest position; *SPPF* – simplified papilla preservation flap; *MPPT* – modified papilla preservation technique; *WKT* – width of keratinized tissue; *EHI* – Early Wound-Healing Index; *p1* – differences between EHI≤2 or EHI≥3; *p2* – differences between EHI ≤3 or EHI = 4; * statistically significant (p <0.05).

Table 3. Odds ratio (OR) as calculated for site-specific characteristics impairing optimal wound healing

Factor	OR	95% CI	*p* value
Phenotype (thin/thick)	0.203	[0.057, 0.721]	0.014

Table 4. Clinical and radiographic parameters as evaluated before and 12 months after surgery

	Presurgery	12 months	p value	Change
PPD (mm)	7.28 [6.96;7.59]±1.23	3.51 [3.29;3.73]±0.86	<0.001	-3.78 [-4.15;-3.40]±1.45
CAL (mm)	8.46 [8.08;8.83]±1.47	4.43 [4.01;4.85]±1.62	<0.001	-4.02 [-4.43;-3.61]±1.59
GR (mm)	1.26 [1.02;1.50]±0.93	1.28 [0.91;1.64]±1.42	0.883	0.03 [-0.31;0.36]±1.31
DD (mm)	5.03 [4.63;5.43]±1.56	0.91 [0.74;1.07]±0.63	<0.001	-4.16 [-4.55;-3.76]±1.53

The means with 95% CI [in brackets] and ± SD of probing values and radiographic measurements of the defects before and 12 months after surgery; PPD – probing pocket depth; CAL – clinical attachment level; GR – gingival recession; DD – radiographic defect depth.

Table 5. Twelve-month change in clinical and radiographic parameters in patients with different early wound healing

	EHI					
Variables	EHI ≤ 2	EHI ≥ 3	p value (≤ 2 or ≥3)	EHI ≤ 3	EHI = 4	p value (≤ 3 or =4)
PPD (mm)	-3.70 [-4.20;-3.20]±1.56	-3.93 [-4.50;-3.35]±1.24	0.576	-3.70 [-4.18;-3.22]±1.57	-3.97 [-4.55;-3.39]±1.12	0.516
CAL (mm)	-4.18 [-4.67;-3.68]±1.53	-3.70 [-4.49;-2.91]±1.69	0.279	-4.16 [-4.65;-3.67]±1.59	-3.65 [-4.46;-2.84]±1.58	0.261
GR (mm)	-0.03 [-0.50;0.45]±1.48	0.13 [-0.30;0.55]±0.92	0.679	-0.05 [-0.49;0.39]±1.43	0.21 [-0.28;0.70]±0.95	0.506
DD (mm)	-4.31 [-4.79;-3.82]±1.53	-3.87 [-4.58;-3.15]±1.53	0.298	-4.36 [-4.86;-3.86]±1.63	-3.66 [-4.25;-3.07]±1.15	0.113

The means with 95% CI [in brackets] and ± SD of probing values and radiographic measurements of the defects before and 12 months after surgery; *PPD* – probing pocket depth; *CAL* – clinical attachment level; *GR* – gingival recession; *DD* – radiographic defect depth.

takes place in the case of large wounds or after the wound margins are spread, a larger amount of new tissue is formed and the healing process takes longer more often accompanied by the formation of tissue scar (reparation) [20]. Appropriate pre-procedural management, proper surgical technique and follow-up care are prerequisites that can promote proper healing. For the periodontal regeneration to take place, necessary conditions for the process must be created during the surgery, such as undisturbed adhesion of the clot to the root surface and then its stabilization [35]. In this context, the role of undisturbed primary healing is well known, especially in the first weeks after GTR [6, 29]. In the present study we evaluated the early healing phase 2 weeks after the GTR treatment of intrabony defects in patients with the Early Wound-Healing Index. Even though primary tension-free flap closure was achieved at the end of surgery in all treated defects, 17 sites (22.87%) healed with secondary intention. The EHI values ranged from 1 to 4. All patients undergoing secondary healing were included in a stringent postoperative care protocol and used a mouthwash solution containing 0.2% chlorhexidine. Over the next 2–4 weeks, all exposed barrier membranes were completely epithelialized.

Morphological features of gingiva, bones and teeth are referred to as gingival phenotypes. The most common are the thin and thick phenotype. The morphology of gingiva remains closely related to the shape and position of teeth and the shape of the alveolar ridge. In the case of the thin phenotype, the gingiva are thin and delicate, the course of the gingival margins is strongly scalloped, with frequent gingival recession and narrowed WKT, the shape of the teeth is triangular, and the contact points are located close to the incisal edges. For the thin phenotype, tissue instability and their high sensitivity to all kinds of injuries, including those related to surgical treatment, are typical. Tissue phenotype might

impinge on surgical treatment outcomes, as flap thickness of 0.8 to 1.2 mm was associated with better prognosis [16]. In the present study, a multivariate analysis revealed that sites with thin phenotype were associated with impaired healing, which means that thick gingival phenotype can be a prerequisite for optimal early wound healing. By the same token, it might be speculated that flap thickness affects its vascularity. Vascularization of the interdental papillae is mainly a network of vascular anastomoses and loops; therefore, both the quantity of soft tissues in interproximal spaces as well as their structure may influence the regulation of blood flow after surgery [20]. The vessels within gingiva on the buccal side run in the apical-coronal direction; therefore, incisions in the papillae can lead to blood flow disorders in these areas [27]. The additional preparation of vertical incisions or periosteal incisions when using barrier membranes may reduce the stabilization and vascularisation of the treatment site and promote secondary healing. Many scientific papers have described the importance and role of various factors affecting early healing following regenerative procedures of bone defects in patients with periodontitis [11, 14, 22]. Variables were evaluated at the level of the patient or tooth (the site undergoing the procedure). Some site-specific variables, such as narrow base of the interdental papilla, presence of interdental contact point and interdental soft tissue crater were associated with impaired healing [11]. In another study, one-rooted teeth, sites with thin phenotype and the presence of gingival recessions were associated with secondary healing [14].

The main objective of the present study was to assess the influence of early healing type of clinical and radiological results of guided tissue regeneration on vertical intrabony defects in 12-month follow-up. Due to the common occurrence of vertical intrabony defects in the course of AgP, this disease entity was selected for the implementation of the GTR surgical procedure. The statistical analysis showed that at 12 months, GTR resulted in significant CAL gain, as well as PPD reduction and DD reduction. No statistically significant differences in the changes of CAL, PPD, GR, and DD were noted between either sites with primary healing (EHI ≤3) or sites with secondary healing (EHI = 4). Similarly, Farina et al. [11] found no significant differences in terms of 6-month CAL gain and PPD reduction at sites with EHI=1 when compared to sites with EHI either >1 or >3. Górski et al. [13] observed that the type of healing did not significantly impact 12-month outcomes after GTR in AgP patients. In a multivariate analysis, the authors demonstrated that intrabony defect morphology (the number of remaining bony walls) might be a predictor of CAL gain, while radiographic baseline defect depth and angle might serve as predictors of changes in bone/graft density evaluated by digital subtraction radiography. On the other hand, Rakmanee et al. [31] analyzed clinical outcomes after GTR versus access flap (AF), in which simplified papilla preservation flaps were used in patients with AgP. While healing at the sites treated with AF was uneventful during early postoperative phase, at 13 out of 18 GTR-treated sites the authors observed membrane exposure and impaired healing. Subsequently, CAL gain was found to be diminished at the sites with membrane exposures at 6 and 12 months post-surgery, compared to those with the non-exposed membranes. A meta-analysis that assessed the effect of membrane exposure on the obtained clinical results of the treatment showed that the sites with impaired early healing had a significantly reduced CAL gain (4.22 [0.15] mm) as compared to the sites with primary healing (4.69 [0.13] mm) ($p < 0.05$) [24]. In addition, it should be remembered that barrier membrane exposure is also associated with an increased probability of bacterial colonization, development of inflammation and local infection, and the mere exposure of the membrane to the oral environment can significantly increase the disintegration of resorbable membranes [23, 26]. In this situation, the barrier membrane ceases to function. Moreover, secondary healing may promote reparation rather than regeneration in periodontal tissues.

Taking into account the number of generated possible post-operative complications, it may be hypothesized that the additional use of biomaterials during periodontal regenerative surgery may interfere with obtaining favorable early healing of soft tissues. In the present study, during all treatment procedures, resorbable collagen membranes were used, which separated the gingival tissue from bone. The rationale for using membranes is that they allow angiogenic and osteogenic cells to migrate into a blood clot. As a result, membranes might markedly undermine the microcirculation of flaps and endanger favorable physiological wound healing. Many years ago it was observed that the use of barrier membrane may be a reason for a temporary impediment to revascularization of mucoperiosteal flaps [39]. Zanetta-Barbosa et al. [40] showed a relationship between impaired blood supply to flaps associated with the use of the barrier membrane and the incidence of impaired healing and wound dehiscences. Jiménez Garcia et al. [18] in their systematic review analyzed the exposure rate in two types of collagen membranes. Spontaneous non-cross-linked membrane exposures ranged from 11% to 32.1%, while for the cross-linked membranes it varied from 12.5% to 56%. The overall relative risk was 1.43, with 95% confidence intervals, with a marginal tendency towards higher exposure in the latter group. In the present study, in all cases, non-cross-inked collagen membranes were used, and the frequency of their exposure amounted to 27.86%. Membrane exposure usually takes place owing to postoperative soft tissue dehiscence and necrosis of a thin flap covering the barrier membrane. Consequently, membrane exposure may result in reduced bone gain [5]. Therefore, the selection of regeneration strategy should be given careful consideration, especially in the case of extensive and non-contained defects. It should be emphasized that surgical procedures implementing membranes are also very demanding from a practical point of view; therefore, technical errors can significantly worsen the effects of treatment [31]. On the other hand, using as an alternative enamel matrix derivatives (Emdogain®, Straumann,

Switzerland) might be associated with fewer post-operative complications [10]. Also, Farina et al. [11] reported that the use of any reconstructive devices might compromise early wound healing, as sites treated with biomaterials (graft only, graft + Emdogain, graft + membrane) showed suboptimal healing (EHI >1). Although various surgical approaches have been tested in combination with a plethora of regenerative materials, none has demonstrated clear advantage over the others [8]. It is obvious that the ideal biomaterial for use in periodontal regenerative treatment has yet to be developed. Unmistakably, the importance of biomaterials with respect to biological responses that regulate and promote wound healing after guided tissue regeneration should further be examined in well-designed future studies.

When interpreting the findings of this study, it is important to take into account some limitations associated with a secondary analysis, for example higher risk of bias. Nonetheless, all data of interest were available for the analysis, as they were collected from all patients. Moreover, the researchers who were interpreting the data took part in the data collection process. Various grafts were used in the treatment of intrabony defects, but only one type of resorbable collagen membrane. Therefore, the effect of the type of defect filling material on soft tissue healing cannot be eliminated, but it seems unlikely. On the other hand, the type of bone substitute material used could be related to the clinical, and especially radiological outcomes obtained after 12 months, due to different radiopacity of materials. In the majority of defects, xenogeneous materials were used. It would definitely be worth conducting a similar study based on a larger group of patients and treatment sites, because the limited number of sites in the present study could be too small for a statistical analysis to show a significant effect of other factors on early healing of soft tissues as well as on long-term outcomes. It would also be valuable to compare whether the type of bone substitute material affects long-term outcomes in the context of the type of early post-treatment healing.

CONCLUSIONS

Within the limitations of this study, it can be said that guided tissue regeneration of intrabony defects in patients with aggressive periodontitis resulted in complete wound closure in the vast majority of sites 2 weeks after surgery. Thick gingival biotype might be a prerequisite for optimal early wound healing. However, the type of early healing (primary/secondary) seems not to affect long-term clinical and radiological outcomes.

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