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EXAMINATION OF THE ROLE OF INTERLEUKIN 1A AND 1B, AND THE APPLICATION OF A-PRF IN MEDICATION-RELATED OSTEONECROSIS OF THE JAW

PhD thesis

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List of abbreviations

AAOMS	American Association of Oral and Maxillofacial Surgeons
ANT	Adenine nucleotide translocase
AMI	Acute myocardial infarction
AP-1	Activator protein 1
A-PRF	Advanced platelet-rich fibrin membrane
ARONJ	Antiresorptive agent-related osteonecrosis of the jaw
ATP	Adenosine triphosphate
BAONJ	Bisphosphonate-associated osteonecrosis of the jaw
BIONJ	Bisphosphonate-induced osteonecrosis of the jaw
BMP	Bone morphogenic protein
BPs	Bisphosphonates
BRONJ	Bisphosphonate-related osteonecrosis of the jaw
cdc42	Saccharomyces cerevisiae proteins
cDNA	Complementary deoxyribonucleic acid
CBCT	Cone beam computer tomography
CC	Cytosine-citozin
COL1A1	Pro-alpha 1 chains of type I collagen encoding gene
COX-2	Cyclooxygenase type-2
CYP2C8	Cytochrome P450 family 2 subfamily C member 8
CP	Chronic periodontitis
C- PRF	Concentrated platelet-rich fibrin
CT	Citozin - timin
CVD	Cardiovascular disease
DAMP	Damage-associated molecular pattern
DNA	Deoxyribonucleic acid
DNB	Denosumab
DRONJ	Denosumab-related osteonecrosis of the jaw/drug-induced osteonecrosis of the jaw
ECG	Electrocardiogram
E-PRF	Extended platelet-rich fibrin
ERKs	Extracellular signal-regulated kinases

FPP	Farnesyl pyrophosphate synthetase
FDP	Fibrin degradation products
FGF	Fibroblast growth factor
GCN5	General control non-repressed 5 protein
GSPMD	Gasdermin D
GTPase	Nucleotide guanosine triphosphate hydrolase enzyme
(HAX)-1	HS-1-associated protein X
HDC	Histidine decarboxylase
HERC4	E3 ubiquitin-protein ligase
HF	Heart failure
H-PRF	Horizontal platelet-rich fibrin
IL	Interleukin
IL-1α	Interleukin 1 α cytokine
IL-1ß	Interleukin 1 ß cytokine
IL-1R	Interleukin 1 receptor
IL-1RacP	IL-1R accessory protein
I-PRF	Injectable platelet-rich fibrin
IRAK4	IL-1R associated kinase 4
JNK	C-Jun N-terminal kinase
KRT18	Type I intermediate filament chain keratin 18 encoding gene
MAPK	Mitogen-activated protein kinases
MC	Myocarditis
MHCII	Major histocompatibility complex class II
MMP2	Matrix metalloproteinase-2
MRONJ	Medication-related osteonecrosis of the jaw
MyD88	Myeloid differentiation primary response protein 88
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLS	Functional nuclear localisation signal
NLRP3	Nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3
NST	Non-surgical treatment/therapy
LRP	LDL-receptor-related protein

ONJ	Osteonecrosis of the jaw
OPG	Osteoprotegerin
OPN	Osteopontin
p300	Histone acetyltransferases CBP
PAMP	Pathogen-associated molecular patterns
PABPC3	Polyadenylate-binding protein 3
PCAF	P300/CBP-associated factor
PDGF	Platelet-derived growth factor
PPARG	Peroxisome proliferator-activated receptor gamma
PRF	Platelet-rich fibrin
Pro-IL-1	Pro form of Interleukin 1 cytokine
PRRS	Pattern recognition receptor
P-C-P	Phosphate – carbon – phosphate bound
Rab	Ras-associated binding (Rab) proteins
Rac	Ras-related C3 botulinum toxin substrate
RANKL	RANK Ligand Inhibitor
RANK	Receptor activator of nuclear factor-kB
RNA	Ribonucleic acid
RMB	Romosozumab
SIRT-1	Nicotinamide adenine dinucleotide-dependent deacetylase sirtuin 1
Smad2	Mothers against decapentaplegic homolog 2
SNP	Single-nucleotide polymorphism
Sp1	Specificity protein 1 (transcription factor)
ST	Surgical treatment/therapy
TGFb1	Transforming growth factor β1 (TGF-1ß)
TGF-1ß	Transforming growth factor β1 (TGF-1ß)
TIR	Toll/Interleukin-1 Receptor
TNFα	Tumor necrosis factor-α
T-PRF	Titanium platelet-rich fibrin
TT	Tirozine - tirozin
VEGF	Vascular endothelial growth factor

1. Introduction

1. 1. Medication-related osteonecrosis of the jaw

1.1.1. Definition and nomenclature

The medication-related osteonecrosis of the jaw (MRONJ) is a relatively rare side effect in the maxillofacial region caused by the application of antiresorptive medications and a certain bone anabolic agent (1).

The main characteristic of the disease is the exposed bone part of the jawbones which may be associated with inflammatory symptoms. In severe cases, the necrotic bone part can extend beyond the alveolar ride and can cause oroantral, oronasal and extraoral fistula or pathologic bone fractures of the lower jaw. The disease diminishes the patients' quality of life because of the strong pain in the jawbone region, halitosis, paraesthesia, fistula discharge, feeding and speech difficulty (1, 2). In severe cases, the MRONJ can give rise to the loss of the jawbone and even to abscess or phlegmon, which are life-threatening complications of the disease.

The disease was first described in 2003 by *Marx* as an avascular necrosis in connection with the administration of two types of bisphosphonates (3). The American Association of Oral and Maxillofacial Surgeons (AAOMS) published a position paper in 2007, which denominated the new disease bisphosphonate-related osteonecrosis of the jaw (BRONJ) and defined its diagnostic criteria, stages and treatment strategies (4). The American Society for Bone and Mineral Research used the term bisphosphonate-associated osteonecrosis of the jaw (BAONJ) for the disease that year (5). Another denomination was bisphosphonate-induced osteonecrosis of the jaw (BIONJ) used by *Marx et al.* and *Kelleher et al.* in their publications (6, 7). In 2009, the AAOMS updated the position paper with the clarification of the disease definition and the supplementation with stage 0 of the stage classification (8).

As a result of the research to reduce the adverse effects of bisphosphonates, a new antiresorptive agent, denosumab, was approved in the treatment of osteoporosis in 2010 (9, 10). That year, *Aghallo* and his colleagues published a case report about a 65-year-old woman treated with denosumab exhibiting symptoms of osteonecrosis of the jaw (11). After that, more and more cases were published that confirmed denosumab's role in the development of osteonecrosis of the jaw (ONJ). Some authors used the term denosumab-

related osteonecrosis of the jaw (DRONJ) to differentiate the cases from BRONJ (12). In 2011, the American Dental Association Council on Scientific Affairs, in light of new knowledge about the disease, extended the diagnostic criteria defined by the AAOMS to all osteonecrosis of the jaw caused by antiresorptive agents (13). The Council on Scientific Affairs introduced a new nomenclature for the disease: antiresorptive agent-induced ONJ (ARONJ) (13).

In 2008, the first case was published about osteonecrosis of the jaw induced by the angiogenesis inhibitor bevacizumab (14). After that, other cases were reported, but in most of them angiogenesis inhibitors were combined with antiresorptive agents in the treatment of the patients (15). Due to angiogenesis inhibitors' role in the development of ONJ, some authors named the disease drug-induced osteonecrosis of the jaw (DRONJ), with the same abbreviation as denosumab-related osteonecrosis of the jaw. (16). In 2014, the AAOMS updated their position paper for the second time. In the new recommendation, the definition of the disease was supplemented and the name of the disease was changed to medication-related osteonecrosis of the jaw (MRONJ) (17). In 2022, the AAOMS published an updated position paper with modified treatment strategies for MRONJ (1).

1.1.2. Medications

The development of MRONJ is linked to the administration of certain antiresorptive and bone anabolic agents. Based on the AAOMS recommendation updated in 2022, the three main types of drugs that can cause MRONJ are bisphosphonates, denosumab and romosozumab (1).

Bisphosphonates (BPs) are antiresorptive drugs that are used for the treatment of diseases that affect bone metabolism. The compound was first synthesised in the middle of the nineteenth century, and its clinical application was first described in the 1960s (18, 19). BPs, as their name suggests, contain two phosphate-carbon bounds, the P-C-P bound, which is the analogue of pyrophosphate (19). Pyrophosphates are natural regulators of bone mineralisation and can attach to the bone mineral (19). BPs, like pyrophosphates, can bind to hydroxyapatite (19). BPs affect several types of cells, such as osteocytes, osteoblast, macrophages, epithelial and also tumor cells, but they predominantly target osteoclasts (19). BPs bring forth the apoptosis of osteoclasts and inhibit osteoclastogenesis (19). Two groups of BPs are known based on their structure, nitrogen-

containing and non-nitrogen-containing/simple BPs. Simple and nitrogen-containing BPs have different intramolecular mechanisms of action. Simple BPs are metabolised intracellularly to nonhydrolyzable analogues of ATP which inhibit the activity of the mitochondrial adenine nucleotide translocase (ANT). ANT has a role in the regulation of apoptosis through permeability transition pores in the mitochondrial membrane (19). Nitrogen-containing BPs exert their effect by blocking the mevalonate/cholesterol biosynthetic pathway (19). The main molecular target of nitrogen-containing BPs is FPP synthetase, but certain agents also inhibit other enzymes of the mevalonate pathway, such as squalene synthase or geranylgeranyl diphosphate synthase (19). The trammel of FPP synthetase inhibits the isoprenylation of GTPases, such as Rho, Rab, cdc42 and Rac. These molecules regulate distinct intracellular signalling pathways that affect osteoclast function and apoptosis (19). BPs are mainly administered in cases of bone metastasis of solid tumors, such as breast cancer, prostate cancer, multiple myeloma and serious cases of osteoporosis (20). These agents are also used in the treatment of rarer diseases, such as osteogenesis imperfecta, Paget's disease and fibrous dysplasia (1).

Denosumab (DNB) is also an antiresorptive agent that was developed in the early 2000s (21). DNB is a human immunoglobulin G2 monoclonal antibody, which can bind to RANKL (21). RANKL is a central molecule in the regulation of bone remodeling. Through the RANKL-RANK-OPG pathway, it can influence osteoclast formation, function and subsist, and therefore bone resorption (21). If DNB is connected to the RANKL, it prevents the RANKL from binding to its receptor RANK, and thus inhibits several intramolecular processes that are necessary for the normal function and survival of osteoclasts, consequently inhibiting bone resorption (21). DNB is used in the treatment of bone metastasis of solid tumors, postmenopausal osteoporosis with high fracture risk and unresectable giant cell tumors of bone (21). DNB is applied also to prevent fragility fractures in patients treated with androgen deprivation therapy or aromatase inhibition therapy because of prostate or breast cancer (21).

Romosozumab (RMB) is a bone anabolic agent that increases bone formation and inhibits bone resorption. RMB is a novel monoclonal antibody against sclerostin (22). Sclerostin interferes with the canonical Wnt – ß catenin signalling pathway that has a central role in bone remodeling (22). In the canonical Wnt – ß catenin signalling pathway, Wnt connects to the Frizzled family receptor and an LDL-receptor-related protein (LRP) coreceptor

(LRP-5 or LRP-6 coreceptor) that inhibits axis assemblies and as a result the phosphorylation of ß-catenin (22). Without phosphorylation, ß-catenin transports to the cell nucleolus and activates the Wnt-responsive genes (22). The activation of Wnt-responsive genes causes osteoblastogenesis and osteogenesis (22). ß-catenin indirectly can inhibit osteoclast differentiation and function through the RANK-RANKL-OPG pathway (22). RMB was accepted in 2019 to treat osteoporosis with a high risk of fragility fractures (1, 22) .

1.1.3. Pathophysiology

Since MRONJ was first described, the pathophysiology of the disease has been under research. However, the exact pathomechanisms and their factors are still unclear. The main, suspected hypotheses in pathophysiology are bone remodeling inhibition, inflammation and infection, angiogenesis inhibition, soft tissue toxicity, immune dysfunction and genetic factors (1, 23). It is assumed that the combination of the processes of several hypotheses plays a role in the development of MRONJ (1, 23).

Bone remodeling inhibition

Bone remodeling inhibition is the main theory in the pathophysiology of MRONJ (1, 23). Antiresorptive drugs are used to treat bone disease which causes bone remodeling imbalance. BPs and DNB affect bone remodeling by decreasing the differentiation and function of osteoclasts and by increasing apoptosis (1, 20). The theory has been confirmed by animal models and preliminary human research (1, 24). The ever-increasing knowledge about the pathomechanism of MRONJ shows that bone remodeling is not only inhibited by the antiresorptive agents' effects on osteoclasts. The antiresorptive agents affect several points and thus modify the complex process of bone remodeling, from different signal molecules to osteoblasts (25). *Wehrhan* and his colleagues in their immunohistochemistry examination found decreased expression of transforming growth factor β1 (TGF-1ß) and Smad -2/3 and increased Galectin-3 staining in BRONJ samples (25). The TGF-1ß has a role in osteoblast differentiation, the RANKL expression in osteoblasts and bone matrix production (26, 27). BMP-2 modified production also affects osteoblast differentiation (28).

Inflammation and infection

The role of inflammation and infection in the pathophysiology of MRONJ has long been known (26, 29). At the advanced stage of MRONJ inflammatory symptoms can be detected, but the healthy bone tissue of the jaw in general resists exposure to oral microorganisms (29). Therefore, it is unlikely that inflammation and infection play a unique role in the pathogenesis of MRONJ. Inflammation and infection together with other factors can lead to the development of MRONJ (1).

Microbiological samples from MRONJ lesions have verified the role of infection in the pathophysiology of MRONJ (26). The main bacteria species detected in microbiological cultures are Peptostreptococcus, Streptococcus, Eikenella, Prevotella, Porphyromonas, Fusobacterium, Bacteroides, Klebsiella, Pseudomonas, Enterococcus, Actinomyces and Eikella (30, 31). Several studies reported that teeth extraction is one of the most common initiating factors in the development of MRONJ (1, 32). Other treatises show that periapical, periodontal and other inflammatory oral diseases are risk factors for the development of MRONJ because the inflammation can modulate the osteoclast function (26).

Angiogenesis inhibition

Angiogenesis and its inhibition may have a role in the pathogenesis of MRONJ (1, 26). BPs directly reduce new vessel and arterial formation (1, 26, 33, 34). Several studies describe diminished vascularity in the bone region and periodontal tissue affected by MRONJ (1, 35). The mucosa also shows microvascular abnormalities in MRONJ cases (35, 36). Based on some studies, the co-administration of antiresorptive and antiangiogenic drugs increases the chance of the development of MRONJ (37). These findings show that angiogenesis also plays a role in the pathophysiology of MRONJ, but this suggestion has not been fully confirmed until now.

Immune dysfunction

Several studies confirm that patients with comorbidities such as diabetes mellitus, malignancies, rheumatoid arthritis or compromised immune status have a higher risk of developing MRONJ if treated with antiresorptive agents (37, 38). Animal studies also verify that treatment with immune-compromised agents such as chemotherapy or steroids combined with antiresorptive therapy increases the chance of developing MRONJ (39).

Soft tissue toxicity

Soft tissue toxicity in the pathogenesis of MRONJ is not generally accepted. However, some authors specify it as a pathophysiological factor. Their main argument is that the first sign of developing MRONJ may be mucosal ulceration (26). Some animal studies confirm this stance. Simon et al. found in their in vitro study that zoledronic acid reduces the production of extracellular matrix protein of oral gingival fibroblasts (40).

Genetic factors

In the last few years, several studies have been conducted which examined the genetic predisposition of MRONJ. Most of them examine defined single-nucleotide polymorphisms (SNPs) of genomic regions that are associated with bone formation, inflammation and angiogenesis. Several SNPs of genes, such as COL1A1, RANK, MMP2, OPG, OPN, TGFb1, CYP2C8, VEGF, PPARG and SIRT-1 showed association with MRONJ (41-43). One of them, SIRT1 is highlighted in the AAOMS Position Paper published in 2022 (1). Clarifying the genetic predisposition of MRONJ requires further investigation, because most of these studies included a small number of patients and examined different SNPs.

1.1.4. Diagnosis and stages

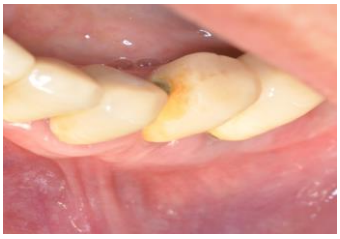
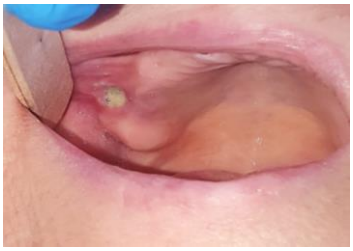
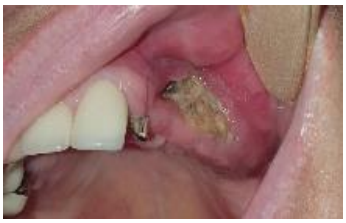
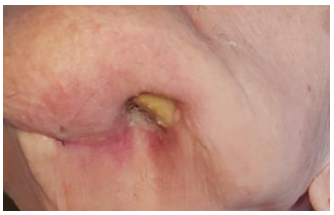
Diagnosis is based on physical examination, patients' health history and imaging tests. AAOMS has defined the diagnostic criteria. The three main diagnostic criteria of MRONJ based on the AAOMS recommendation last updated in 2022 are listed in **Table 1.**

Table 1: The diagnostic criteria of MRONJ
Own table based on AAOMS Position Paper Updated in 2022 (1)

Diagnostic criteria of MRONJ	
1.	Antiresorptive treatment alone or in combination with immune modulators or antiangiogenic drugs in patients' anamnesis
2	Exposed bone or bone that can be detected across an intraoral or an extraoral fistule in the maxillofacial region that has consisted for more than eight weeks
3.	No radiation therapy to the jaws in patients' anamnesis and the metastatic disease to the jaws is ruled out

AAOMS has also established the stage classification of the disease. The symptoms and the clinical and radiographical findings of each stage are shown in **Table 2.**

Table 2: Clinical and radiological findings in different stages of MRONJ
Own table based on AAOMS Position Paper updated in 2022 with own photos (1)

Clinical and radiological findings in different stages of MRONJ		
	Clinical findings	Radiological findings
Stage 0 	No clinical evidence of necrotic bone Spontaneous tooth loosing not caused by chronic periodontal disease Intraoral or extraoral mucosal swelling	Alveolar bone loss or resorption Changes to the trabecular pattern of bone No new bone in extraction sockets Osteosclerotic regions affect the alveolar bone and/or the basilar bone. Thickening of periodontal ligament
Stage 1 	Exposed and necrotic bone or fistula No signs of inflammation/infection	Alveolar bone loss or resorption Changes to the trabecular pattern of bone No new bone in extraction sockets Osteosclerotic regions affect the alveolar bone and/or the basilar bone. Thickening of periodontal ligament
Stage 2 	Exposed and necrotic bone or fistula Signs of inflammation/infection	Alveolar bone loss or resorption Changes to the trabecular pattern of bone No new bone in extraction sockets Osteosclerotic regions affect the alveolar bone and/or the basilar bone. Thickening of periodontal ligament
Stage 3 	Exposed and necrotic bone or fistula Signs of inflammation/infection One or more of the following findings: -Necrotic bone extending beyond the alveolar bone -Pathologic fracture of mandible -Extraoral fistula -Oro-antral/oral-nasal communication.	Alveolar bone loss or resorption Changes to the trabecular pattern of bone No new bone in extraction sockets Osteosclerotic regions affect the alveolar bone and/or the basilar bone. Thickening of periodontal ligament

1.1.5. Treatment strategies

MRONJ can be treated with non-surgical (NST) and surgical (ST) methods. According to the AAOMS recommendation updated in 2022, the application of both nonoperative and operative therapies is considered professionally founded and acceptable in each stage of the disease depending on the patients' factors and surgical judgment (1).

1.1.5.1. Non-surgical therapy

Non-surgical therapies are applied in cases where the patients have serious comorbidities that do not allow surgical treatment (1). They are also effective in the early stages of MRONJ and applied in addition to surgical therapy (1). Several publications corroborate the success of NOT (44, 45).

NST is acceptable in all stages of MRONJ but leads to a cure only in very early stages (1, 46). In advanced stages, NST is not effective, it is used to stabilize the disease (1, 47). In addition to surgical treatment, the different NST therapies are useful (1, 47). The choice between nonsurgical and surgical therapy is in every case a patient-specific and individual one, based on the patients' and the disease factors (1).

Conventional NST consists of local wound care, antimicrobial rinses and removal of well-formed sequestrum in stage 1. In stage 2 these are combined with systemic antibiotics and pain control, in stage 3, as an additional measure, the antibiotics may be administered intravenously (1). Several adjunctive conservative therapies have been tested to enhance the treatment efficacy of MRONJ. Numerous publications demonstrate the effectiveness of these adjunctive therapies in the treatment of MRONJ, but no randomised-controlled studies and meta-analyses proved this effectiveness (1). Because of this, the last AAOMS recommendation did not suggest administering these treatments alone, only as additional therapy (1). These adjunctive treatments include hyperbaric oxygen therapy, ozone therapy, vitamin E and pentoxifylline administration, teriparatide usage, laser therapy etc (48).

1.1.5.2. Surgical therapy

Numerous studies demonstrate the high success rate of ST in the treatment of MRONJ (49-51). According to these publications, the usage of ST improves the outcome and progression of the disease and ameliorates patients' quality of life (50, 52). Other benefits of ST are the mucosal coverage of the earlier exposed/necrotic jaw area and the chance

to resume antiresorptive/bone anabolic treatment as early as possible (53). An additional advantage is that the histological examination supports the diagnosis and disqualifies the jaw metastasis. Surgical therapy is acceptable in 1-3 stages of the disease (1). Surgical therapy means the removal of the necrotic bone area down to vital, bleeding bone surfaces and primarily wood closure. Depending on the stage of the disease, in the case of the upper jaw alveolectomy or partial maxillectomy is performed to remove the necrotic bone area, while a marginal or segmental resection is carried out in the case of the lower jaw (1). The AAOMS recommendation updated in 2022 suggests that ST is effective at all stages of MRONJ, including if diagnosed at an advanced stage, and both physical examinations and imaging tests confirmed that it has a favourable effect on prognosis (1). The effectiveness of ST is higher than NST, even so, 65-86% of the necrotic lesions are recovered for the first operation, and in the case of healing the chance of recurrence is about 11-45% (54, 55). Therefore, several additional procedures – regenerative materials, and conservative methods - have been tested to intensify the effectiveness of surgical therapy (53).

1.1.6. Risk and prognostic factors of MRONJ

Since the first description of the disease, several investigations have been published that have investigated the risk factors of MRONJ. The results of these examinations are diverse and many factors have been related to the development of disease. These factors are among others the chemo- or hormonal therapy, the admission dosage of the antiresorptive drug, breast cancer from underlying diseases, corticosteroid treatment, diabetes mellitus, periodontal disease and tooth extraction (56-58).

The recurrence rate of MRONJ has been described in various studies as between 11% and 45% depending on the type of surgery (55, 59-62). Impaired healing and recurrences after surgical therapy have been explained by insufficient removal of the necrotic bone and coverage of the remaining bone surface, and inflamed mucosa due to postoperative dehiscence (60-64). Even with the most appropriate surgical therapy, relapses are inevitable due to the effects of antiresorptive agents on bone remodeling (62, 65). Several studies have examined potential risk factors affecting the prognosis after surgical therapy (60, 62, 66). Based on publications, the possible factors affecting prognosis among others are the size of the necrotic bone part, diabetes mellitus, antiestrogen therapy, the total

dose of bisphosphonate administered, the type of bisphosphonate administered, and smoking (60, 62, 66-68).

1.2. Interleukin-1α and β

1.2.1. Interleukin-1 cytokine family

Interleukin-1α and β (IL-1α and IL-1ß) are two of the 11 cytokines comprising the interleukin-1 family (69). These cytokines have a central regulatory role in the function of the innate immune system, in response to pathogens and haematopoiesis (69). These substances, including pyrexin and leukocytic pyrogen, were first described in the 1940s by *Menkin and Beson*, who found in their experiments that the supernatants of rabbit neutrophils give rise to fever (69). The Second International Lymphokine Workshop held in Switzerland in 1979 introduced the terminology interleukin and named these molecules interleukin-1 (69, 70). The first identification and description of the interleukin-1 precursor cDNA sequence was in 1984 (69).

The members of the interleukin-1 cytokine family can be classified into three subgroups: interleukin-1, interleukin-18 and interleukin-36 (70). Interleukin-1α, interleukin-1β and interleukin-33 belong to the inetrelukin-1 subgroup (70). All of these molecules are pro-inflammatory cytokines (70).

1.2.2. Interleukin-1α (IL-1α)

The IL-1A gene encodes IL-1α. The IL-1A gene is transcripted differently in distinct cells of the human body (71). IL-1α is continuously expressed in keratinocytes in the skin, type 2 epithelial cells in the lung, the epithelium of the entire gastrointestinal tract, endothelial cells in blood vessels and astrocytes in the brain (72). In these endothelial and epithelial cells, the transcription is constitutive, and in these cells, transcription factor Sp1 mediates the process (71). In haematopoietic and immune cells, the transcription is stimulated by several molecules, such as Toll-like receptor agonists, hormones and inflammatory cytokines (71). AP1 and NF-κB transcription factors mediate the stimulated transcription (71). The transcription and translation lead to the synthesis of pro-form IL-1α molecules (71). These pro-form molecules can shuttle to the nucleus or cytosol, depending on the cell type and the cell-specific regulating process (71). The exact regulating mechanisms in different cell types are mostly unknown. One of the suspected mechanisms is that the regulation is processed with a specific signal structure, a functional nuclear localization

signal (NLS) (71). Pro-IL-1α contains NLS in the N-terminal (71). If the NLS is masked with IL-1R2, it causes the retention of IL-1 in the cytosol. In the absence of masking, the pro-IL-1 is shuttled to the nucleus (71). Another mechanism is that HS-1-associated protein X (HAX)-1 connects to pro-IL-1α and facilitates its nuclear delivery (71). Pro-IL-1α makes contacts in the nucleus among others with histone acetyltransferases p300, P300/CBP-associated factor (PCAF) and GCN5, and regulates the gene expression, such as IL-8 transcription (71). Accordingly, the IL-1α can shuttle to the nucleus and operate like a transcription factor, it can connect to its cell membrane receptor, IL-1R, and can stimulate signal transduction processes, consequently, some authors named it dual-function cytokine (71). Pro-IL-1α also has a membrane-bounded, specific form that is stimulated by inflammation in endothelial cells of the innate immune system (71, 72). The pro-IL-1α and the cleaved IL-1α are also active forms and can operate the IL-1R1 receptor (72). The pro-IL-1α is cleaved by proteases to mature form, IL-1α (72) (71). These proteases, such as caspase-1, y calpain, chymase, elastase and granzyme B, can be found both extracellularly and intracellularly (71). The IL-1α is secreted during the process of pyroptosis induced by an inflammatory process or cell death (71). The inflammatory process or cell death leads to inflammasome formation and activation through microbe-derived pathogen-associated (PAMPs) or damage-associated (DAMPs) detected by pattern recognition receptors (PRRs) (73). The activation of inflammasomes induces caspase-1 activation. The activated caspase-1 cleaves numerous proteins, in addition to pro-IL-1α, also IL-1β, gasdermin and the D N-terminal fragment of GSDMD oligomerizes (73). The latter two bring forth explosive cell death through the increase in intracellular osmotic pressure and cell swelling (73). During the explosive cell death, IL-1α is released from the cell. Caspase-1 can also cleave IL1R2 in pro-IL-1α-IL1R2, which contains a connected form of pro-IL-1α. This process causes the dissociation of the complex and increases the bioavailability of pro-IL-1α (72, 73). The pro-IL-1α-IL1R2 complex also plays a regulatory role in the immune process, because the binding form of pro-IL-1α can't be released from the cell (72, 73). After the IL-1α is released from the cell, it can bind to its receptor, IL-1R. The binding of IL-1α to its receptor brings forth structural changes and the IL-1RacP, the transmembrane signal transduction subunit of IL-1R1, can bind to IL-1R1 and form a complex (73). The complex contains a TIR domain intracellularly. MyD88 binds to the IL-1R1 – IL-1RacP complex through the TIR

domain, activating the phosphorylation cascade that activates NF-κB, activator protein-1 (AP-1), c-Jun N-terminal kinase (JNK), p38 and other mitogen-associated protein kinases (MAPKs), extracellular signal-regulated kinases (ERKs) and interferon-regulating genes (72, 73). Through these molecules, IL-1α exerts its effect on the target cell, for example, evoking the secretion of TNFα, IL-6 and cyclooxygenase type-2 (COX-2) in epithelial and haematopoietic cells. In addition, IL-1α, mediated by the molecules listed above, induces the production of IL-2, interferons, chemokines, prostaglandins and adhesion molecules, activates T-helper cells and stimulates the maturation and clonal expansion of B cells (72). The activation of IL-1R through these molecules further enhances the release of IL-1α. The membrane-associated form of pro-IL-1α is activated by extracellular protease and through its receptor, which causes the induction of T cell proliferation and chemokine secretion (72, 73).

1.2.3. Interleukin-1β (IL-1ß)

The IL-1β is expressed in a few types of cells, such as macrophages, dendritic cells, neutrophils, B lymphocytes, natural killer cells and keratinocytes induced by inflammatory signals (71, 74). The activation of pattern recognition receptors (PRRs) by pathogen-derived products and molecules from damaged cells triggers the expression of IL-1ß (74). The IL-1B gene encodes it (74). AP1 and NF-κB transcription factors regulate the transcription of the IL-1B gene (71). During the translation process, a pro-form of IL-1ß, pro-IL-1ß is produced. Pro-IL-1ß is biologically inactive and intracellular processing is needed for activation (72). Pro-IL-1ß is cleaved to active IL-1ß-form by the caspase-1 protease (72). The IL-1ß release from the cell is similar to IL-1α. Activated caspase-1 induces explosive cell death and during this process, IL-1ß is released from the cell. IL-1β can bind to its receptor, IL-1R, with the same kinetic as IL-1α (72). The biological effect and effectiveness of IL-1ß are also the same as IL-1α (73). The IL-1ß binding to IL-1R causes the same molecular and structural changes as IL-1α (73). IL-1ß through the IRAK pathway activates the NK-κB and also incites the production of transcription factors, MAPKs, ERKs and interferon-regulating genes, which are listed in the previous section concerning IL-1α (71, 72). IL-1ß stimulates the TNFα, IL-6 and COX-2 secretion of haematopoietic and epithelial cells and also incites the other molecules' production, listed above in connection with IL-1α (72). The activation of IL-1R also promotes the IL-1ß secretion (72).

1.2.4. The role of interleukin-1α and β in diseases

Numerous studies demonstrate the association between interleukin-1α and β malfunction and various diseases, such as autoimmune disorders, lung disease, cardiovascular diseases, musculoskeletal disorders and cancers. Due to compliance with the formal requirements of the thesis, only a few will be presented.

Musculoskeletal disease

Myositis, such as dermatomyositis and polymyositis are inflammation-mediated diseases of the muscles. Several pro-inflammatory mediators participate in the pathomechanism of the disease, including 1L-1α (72). Clinical experiences confirm IL-1α's role in myositis: high expression of IL-1α causes persistent muscle weakness (72).

Several autoimmune rheumatic diseases are associated with overexpression of IL-1α or IL-1ß, such as osteoarthritis, rheumatoid arthritis, gout arthritis, ankylosing spondylitis, psoriatic arthritis, adult-onset and systemic-onset juvenile idiopathic arthritis (Still's disease) (75-77).

Atherosclerosis and cardiovascular disease

IL-1α and β have a central role in the pathophysiology of atherosclerosis, so in the development of coronary-artery disease, acute myocardial infarction and brain injury (71, 77). Macrophages, lipids and cholesterol crystals assemble in the atherosclerotic plaque. The oxidized LDH stimulates IL-1α and IL-1β production in macrophages (77). During the maturation of the plaque, necrosis and apoptosis also induced IL-1α and IL-1β expression (77). IL-1 overexpression leads to vasculitis near the plaque (71). Cholesterol crystals can also promote IL-1ß production through NLRP3 inflammasome direct activation (73, 77). Several studies have proven that IL-1 cytokines-associated mechanisms take part in the development of heart diseases, such as myocardial infarction (AMI), heart failure (HF), myocarditis (MC). During AMI, the NLRP3 inflammasome is activated not only in myocardial cells, but in all heart cells (73). The activation of the NLRP3 inflammasome stimulates the release of IL-1ß and IL-1α, which intensifies the immune response (73). The inflammation causes vasoplegia and cardiac dysfunction indirectly (73). Interleukin-1 regulates cardiac contractility directly and participates in atherothrombosis formation (73). Through these mechanisms, it affects the development and course of the disease (73). The role of Interleukin-1 in HF is demonstrated also by

early clinical trials that have used *anakinra*, an IL-1R1 human recombinant antagonist, in stable chronic and systolic HF, and acute decompensated systolic HF Viral infection caused acute, idiopathic MC associated with IL-1 production (73).

Infections

Interleukin-1α and β have a central role in initiating and stimulating the immune system in the immune response process to pathogens and in bacterial, viral, fungal and protozoon infections (71).

Interleukin-1α instigates the immune response in the case of several bacteria, such as *Staphylococcus aureus* and *Chlamydia trachomatis*, and stimulates neutrophilia in a defence reaction in the case of *Legionella pneumophila* and *Haemophilus influenzae* infections. Interleukin-1β is a mediator in the immune response to *Streptococcus pneumonia, Citrobacter rodentium* and *Francisella novicida* (71). Also, Interleukin-1α and β expression is increased in *Mycobacterium tuberculosis* infections (71). IL-1α through the stimulation of neutrophil granulocytes participates in the defence mechanisms against adenovirus. Out of fungal infections, *Candida albicans* infections intensify the production of IL-1α, and *Cryptococcus neoformans* and *Apsergillus fumigatus* also stimulate the expression of IL-1α and IL-1β (71).

Oral disease

According to investigations, Interleukin-1α and β elevated serum levels are associated with numerous oral pathologic conditions. Interleukin-1β has a role in the pathogenesis of chronic periodontitis (CP) (78). Elevated IL-1β level was detected in saliva, gingival crevicular fluid and soft tissue in CP (78). Elevated IL-1β levels cause increased local blood flow, leukocyte and neutrophil infiltration in the affected area, and bone resorption (78). Increased IL-1α and IL-1β expression was also identified in chronic pulp inflammation and periapical granulomas (79, 80). IL-1β is also connected to the development and progression of temporomandibular disorders (81). *Brailo et al.* found in their study that the IL-1β salivary level is significantly higher among oral cancer patients than in the control group or patients with leucoplakia (82). According to a study conducted by *Asensi et al.*, the serum levels of IL-1α in patients with chronic osteomyelitis were significantly higher than in control patients (83).

1.2.5. Inetrleukin-1A and 1B genes and their single nucleotide polymorphisms

IL-1α is encoded by the Interleukin-1A gene (IL-1A) and IL-1ß is encoded by the Interleukin-1B gene (IL-1B) (84). IL-1A and IL-1B genes are located at the long arm of chromosome 2 (2q14), in the same gene cluster with genes encoding seven other IL-1 cytokines (62, 84). The gene size of IL-1A is 14 kb and contains seven exons and six introns. The IL-1B gene size is 7,5 kb and contains seven exons and six introns (84). The sequence homology is less than 26% in the two genes (84).

Until now, 148 single nucleotide polymorphisms (SNPs) of the IL-1A gene and 144 of the IL-1 B gene have been identified (84). The SNPs are base pair variations at a single position in the DNA sequence that occur in more than 1% of the population. SNPs are located in protein-coding and also in non-coding DNA regions (84). The SNPs in the protein-coding regions cause different bases contents of the transcribed mRNA (84). The SNPs in the non-protein-coding regions can interfere with the transcription process through the loss or alteration of binding sites for RNA and DNA binding proteins (84). The SNPs through structural changes in the mRNA induce minor amino acid changes in translated proteins, which can lead to changes in protein processing, maturation and posttranslational modification, and consequently in protein function and interactions with other proteins as well. SNPs can influence the rate and level of transcription and translation by changing binding sites for RNA and DNA binding proteins (84). The SNPs of IL-1A and IL-1B genes can change cytokine production and interactions, which can lead to an altered immune response to pro-inflammatory signals (84). The enhanced immune response can lead to autoimmune disease, chronic inflammations and cancers, and the deprived immune response can predispose patients to infections and sepsis (84).

One of the most investigated SNP of the IL-1A gene is rs18000587 at position −889 C>T in the promoter region. Studies found no statistically significant differences in the levels of mRNA in subjects with TT genotype compared to CT or CC genotypes; however, in the serum levels of IL-1α a disparity was detected between genotypes (CC 6.8 pg/mL, TT 36 pg/mL) (84).

In addition to the two promoters' SNPs, rs1143627 at position −31 T>C and rs16944 at position C>T, the most examined SNP of IL-1ß is rs1143634 at position +3954 (84). The rs1143634 SNP does not cause changes in the amino acid sequence or the serum level of

IL-1ß, but studies show its correlation to several diseases. rs1143634 presence in the healthy Caucasian population is T allele 0.73 and C allele 0.27 (84).

The SNPs of IL-1A and IL-1B genes have a role in several diseases (85). Rs1800587, the SNP of the IL-1A gene, is related to Alzheimer's diseases, obesity, sepsis, lumbar disc disease, chronic osteomyelitis and chronic periodontitis (83, 85-87). Rs1143634, the SNP of the IL-1B gene, is possibly associated with lung cancer, chronic periodontitis, Systemic Lupus Erythematosus, and sepsis (83, 85-87).

1.3. Platelet – rich fibrin

1.3.1 Brief history of platelet concentrates and platelet-rich fibrin

Platelet-rich fibrin (PRF) is a platelet concentrate (88). The platelet concentrates are the processed, liquid part of peripheral blood that contain platelets in higher concentration than the peripheral blood (88). These concentrates were named "platelet-rich plasma" by haematologists and were administered intravenously to patients with thrombocytopenia (89). From the 1970s, these concentrates were applied as „fibrin glues" in surgical procedures to stimulate wound healing (90, 91). The first platelet-rich plasma gels were created by modifying the production process, which already contained a high concentration of platelets (90, 91). These products have been widely used in general surgery, neurology, ophthalmology and plastic surgery (92, 93). *Marx et al.* applied the platelet-rich plasma first in oral surgery procedures and published their experiences in 1998 (94). In the early 2000s, a new production process was developed by *Choukroun et al.* that was significantly different from the previous one and resulted in the formation of a strong fibrin network in the product (95). The new products were named PRF and because of the new procedure technique, they mean the second generation of platelet concentrate (95).

1.3.2. Application of platelet-rich fibrin

PRF is applied in several medical specialities, such as oral and maxillofacial surgery, periodontology, orthopaedic surgery, and plastic surgery to improve wound healing and regeneration of the surgical site (96-100). PRF supports tissue repair initiation through containment growth factors, cytokines and adhesion proteins that have a role in the hemostatic cascade, connective tissue synthesis, and angiogenesis (101, 102). PRF also

improves the natural healing potential of hard and soft tissues by containing growth factors, such as VEGF, FGF, PDGF, and modulating pain and inflammation (103).

1.3.3. Types of platelet-rich fibrin

In the last few years, the PRF process protocol has been modified to improve the function and receive a PRF with consistency and composition appropriate to the treatment procedure. As a result of the modifications, several various products have been created with different biology and potential applications. The various products are shown in **Table 3.**

Table 3: Types of PRFs, own table based on (98, 104-111)

	Type of PRF	Preparation technic	Advantages
Leukocyte and platelet-rich fibrin (L-PRF)	"Conventional" L-PRF	3,000 rpm for 10 minutes	Compared to PRP: no anticoagulants strong fibrin clot with blood cells
	Advanced PRF (A-PRF)	1,500 rpm/1,300 rpm for 14 minutes	Compared to L-PRF: more viable blood cells the higher total amount of growth factors
	Advanced PRF plus (A-PRF+)	1,300 rpm for 8 minutes	Compared to L-PRF/A-PRF: a larger amount of growth factors higher number of white blood cells
	Injectable PRF (I-PRF)	700 rpm for 3 minutes	Compared to L-PRF: injectable form more growth factors early on
	Horizontal-PRF (H-PRF)	700 rpm for 8 minutes horizontal angle	Compared to L-PRF: four times greater leukocyte concentrations
	Concentrated PRF (C-PRF)	2000 g for 8-minute	Compared to L-PRF: boosts cell and growth factor yield
	titanium-PRF (T-PRF)	2,800 rpm for 12 minutes titanium tubes	Compared to L-PRF: last longer in tissues
	extended PRF (E-PRF)	700 g for 8 minutes heat 75 °C for 10 minutes cool for 1-2 minutes	Compared to L-PRF: prolonged resorption profile
Leukocyte-poor or pure platelet-rich fibrin		specific protocol	Compared to PRP: denser, more stable, and more efficient fibrin matrix

2. Objectives

MRONJ is an adverse drug reaction of antiresorptive therapy (1, 112). MRONJ is developed in only 0.01 - 19% of the patients receiving antiresorptive therapy (1, 57, 113). The suppositional risk factors are among others the therapeutic indication of antiresorptive treatment, the type and duration of the antiresorptive therapy (1). Presumably, in the pathophysiology of the disease, among others, bone remodeling inhibition, infection and inflammation, and genetic factors play a role (1). The prognosis of MRONJ is unfavourable, the recovery is only achieved in a maximum 85-86% of the cases and the recurrence rate is high (59). Numerous factors are associated with recurrences, such as the type of antiresorptive therapy administration, the MRONJ stage, (68, 114). Despite the results of several studies published in the past decades, the exact pathophysiology, the risk and prognostic factors and the infallible treatment procedure ensuring complete healing are still unknown.

The subject of my doctoral studies is examining the factors that contribute to the development and prognosis of MRONJ and investigating the effectiveness of possible supplementing procedures in surgical therapy. During my doctoral studies, I conducted two main clinical studies, which have concluded with results.

2.1. Hypothesis and aims of the study examining the role of single nucleotide polymorphisms of Interleukin 1A and 1B in MRONJ

The study hypothesis is based on the knowledge of MRONJ gained from the publications summarised above. It is suggested that a genetic variation may have a role in MRONJ pathomechanism, which is involved in both the inflammatory process and bone remodeling (1). Interleukin 1-α and ß are the central initiator and regulator molecules of the innate immune system and affect bone remodeling (69). Increased IL-1 levels are identified in several diseases, among others in inflammatory diseases affecting the bone or having bony components, such as chronic osteomyelitis and periodontitis (71, 73, 83, 87). Some studies have proven the role of IL-1A−889 (rs18000587) and IL-1B+3953 (rs1143634) in chronic osteomyelitis and periodontitis, which diseases' pathomechanisms are similar to MRONJ at several points (87, 115).

The study hypothesis is that the defined SNPs of IL-1A and IL-1B genes have a role in the development and prognosis of MRONJ. The aim of the study is the examination of

the role of the IL-1A−889 (rs18000587) and IL-1B+3953 (rs1143634) SNPs in the development and prognosis of MRONJ:

- *Aim I/1:* Examine the role of defined SNPs in the development of MRONJ
- *Aim I/2:* Examine the role of defined SNPs in the prognosis (based on stage improvement, healing, recurrence, and recurrence number) of MRONJ
- *Aim I/3*: Examine the defined SNP's effect on the necrosis features
- *Aim I/4:* Examine the defined SNP's probable effect on the prognosis of MRONJ combined with anamnestic and therapeutic data

2.2. Hypothesis and aims of the study examining the A-PRF application in surgical therapy of MRONJ

The study hypothesis is also based on the information published about MRONJ. In the next 10-15 years, an increasing number of published studies and systematic reviews have shown that surgical treatment is more successful than conservative therapy (1, 49, 51). However, surgical therapy leads to healing in only a maximum 85% of the cases (116). For this reason, some additional procedures have been tested as a supplement treatment to surgical therapy to improve its effectiveness, such as the application of regenerative materials, hyperbaric oxygen therapy and lower-level laser therapy (59).

One of the regenerative materials whose application may ameliorate the surgical therapy of MRONJ is platelet-rich fibrin (PRF). PRF is a second-generation thrombocyte concentrate (61, 95). The framework of PRF is formed by a 3-dimensional fibrin network with imbedded thrombocytes, leukocytes and stem cells (61, 95). PRF also includes several growth factors, cytokines and proteins. PRF supports immunity and angiogenesis on the surgical side by stimulating wound healing (95, 117). The PRF is applied in several oral and maxillofacial procedures to accelerate wound recovery (118).

The study hypothesises that the application of PRF improves the effectiveness of surgical therapy in terms of wound healing and the prognosis of the disease. The aim of the study is to explore the effect of supplementing the surgical therapy with the membranous form of PRF (A-PRF) on wound healing and on the prognosis of MRONJ:

- *Aim II/1:* Examine the A-PRF usage effect on the prognosis (based on stage improvement, healing, recurrence, and recurrence number) of MRONJ
- *Aim II/2:* Examine the effect of the necrosis features and the anamnestic and therapeutic data on the outcome of the PRF-supplemented surgical therapy

3. Methods

3.1. Study design

3.1.1. Study design for examining the role of SNPs of Interleukin 1A and 1B in MRONJ

The first investigation is an ambidirectional cohort study which examines the role of the defined polymorphisms (IL-1A−889 – rs18000587; IL-1B+3953 – rs1143634) of the IL-1A and 1L-1B genes in relation to MRONJ. The study complies with the Helsinki Declaration, local laws and data protec tion regulations. The investigation was approved by the Scientific and Research Ethics Committee of the Scientific Council for Health (Approval number: 2507 2/2016) (62).

The role of SNPs in the development of MRONJ *(Aim I/1)* is examined by comparing the occurrence of SNPs in the patient and the control group. The role of SNPs in the MRONJ prognosis *(Aim I/2)* is examined in patients treated with surgical therapy according to stage improvement, healing and recurrences after the operation. The occurrence of SNPs is compared in the given patient groups, stage improvement – no stage improvement, healing – no healing and recurrences – no recurrences and recurrence number to define the role of SNPs in prognosis. The possible effect of the determined SNPs on the necrosis features *(Aim I/3)* is also investigated, by examining the occurrence of the defined SNPs in certain categories of the necrosis features. The examined features of necrosis are shown in **Table 8**. The defined SNPs' probable effect combined with anamnestic and therapeutic data on the development and prognosis of MRONJ *(Aim I/4)* are also investigated. The anamnestic and therapeutic data are shown in **Table 4**.

The SNPs are analysed separately and in combination in all four aims of the study as follows:

A, the occurrence of the SNP (IL-1A 889 C/T, IL-1B+3953 C/T) on any copy of any of the two genes is examined (any of the examined SNPs occurred/ none)

B, the occurrence of the examined SNP (IL-1A-889 C/T) on any copy of the IL-1A gene only (no occurrence of the examined SNP IL-1B+3953 C/T on any copy of the IL-1B gene), the examined SNP (IL-1B+3953 C/T) on any copy of the IL-1B gene only (no occurrence of the examined IL-1A-889 C/T on any copy of the IL-1A gene)/ the examined SNPs (IL-1A-889 C/T, IL-1B+3953 C/T) on any copy of the IL-1A and IL-1B genes

C, the examined SNP (IL-1B+3953 C/T) of IL-1B gene is carried on all or one or none copies of the gene (homozygous for the SNP [IL-1B+3953T, IL-1B+3953T], heterozygous for the SNP [IL-1B+C3953, IL-1B+3953T], homozygous for the wild type allele [IL-1B+C3953, IL-1B+C3953])

D, the examined SNP (IL-1A-889 C/T) of the IL-1A gene is carried on all or one or none of the copies of the gene (homozygous for the SNP [IL-1A-889T, IL-1A-889T], heterozygous for the SNP [IL-1A-889T, IL-1A-C889], homozygous for the wild type allele [IL-1A-C889, IL-1A-C889])

E, the examined SNPs (IL-1A-889 C/T, IL-1B+3953 C/T) of the genes are carried on all or one or none of the copies of the given genes, the nine combinations are:

- IL-1A 889T, IL-1A-889T, IL-1B+3953T, IL-1B+3953T
- IL-1A-889T, IL-1A-889T, IL-1B+C3953, IL-1B+3953T
- IL-1A-889T, IL-1A-889T, IL-1B+C3953, IL-1B+C3953
- IL-1A-889T, IL -1A-C889, IL-1B+3953T, IL-1B+3953T
- IL-1A 889T, IL-1A-C889, IL-1B+C3953, IL- 1B+3953T
- IL-1A-889T, IL-1A-C889, IL-1B+C3953, IL-1B+C3953
- IL-1A-C889, IL-1A-C889, IL-1B+C3953, IL-1B+C3953
- IL-1A-C889, IL-1A-C889, IL-1B+3953T, IL- 1B+3953T
- IL-1A-C889, IL-1A-C889, IL-1B+3953T, IL-1B+3953T

3.1.2. Study design for examining the A-PRF application in MRONJ

The second investigation is a randomized control trial in which the effect of the membranous form of platelet-rich fibrin (A-PRF) is examined in MRONJ after surgical therapy. The study observes the Helsinki Declaration, local laws and data protection regulations. The investigation was approved by the Scientific and Research Ethics Committee of the Scientific Council for Health (Approval number: 17041-4/2019) (61) . The effect of the application of A-PRF on the prognosis of MRONJ is examined by comparing the prognostic features, such as stage improvement, healing and recurrences in a patient group treated with traditional surgical therapy and a patient group treated with A-PRF complemented surgical therapy *(Aim II/1)*. The effects of the necrosis features and the anamnestic and therapeutic data are also investigated on the outcome of the PRF-supplemented surgical therapy *(Aim II/2)*. The tested anamnestic and therapeutic data are shown in **Table 9 (61)**.

3.2. Patient and control group

3.2.1. Patient and control group for examining the role of SNPs of Interleukin 1A and 1B in MRONJ

Both investigations involved patients over 18 years of age, having reached the age of discretion and treated at the Department of Oro-Maxillofacial Surgery and Stomatology, Faculty of Dentistry, Semmelweis University. All patients received detailed information about the procedures and signed informed consent to participate in the study.

To clarify the role of the defined SNPs in the development of MRONJ *(Aim I/1)* by comparing the occurrence of SNPs in the patient group and the control group. Patients treated with MRONJ were included in the patient group. The diagnosis of MRONJ was based on the criteria defined by the recommendations of AAOMS (17) . The control group consisted of patients with no MRONJ. Physical examination or imaging tests showed no signs or symptoms of necrosis in any of these patients. The control group was homologous with the patient group in terms of age and sex. The *Aim I/2, Aim I/3 and Aim I/4* are examined only in the patient group. Exclusion criteria were if the patient refused the surgical treatment and was unwilling or unable to return for required follow-up visits.

3.2.2. Patient and control group for examining the A-PRF application in MRONJ

The second investigation included patients who were diagnosed with MRONJ and were treated with surgical therapy for the disease. Further inclusion and exclusion criteria are indicated in **Table 4**. To clarify the effect of additional A-PRF in surgical treatment, the traditional and A-PRF-supplemented surgical therapy were compared. The patients were divided into two groups. The first group was treated with traditional surgical therapy, and in the second group, A-PRF-supplemented surgical therapy was applied.

Table 4: Inclusion and exclusion criteria of patients in the secund study

Inclusion criteria	**Exclusion criteria**
diagnosed with MRONJ	refuses surgical therapy or application of A-PRF
surgical therapy is the first choice in the treatment	not willing to and unable to return for required follow-up visits
the general condition of the patient allows for surgical intervention	receiving chemotherapy around the expected time of operation
preoperative CBCT shows that there remain at least 2-sided bone pockets after the removal of the necrotic bone part, which means marginal resection or alveolectomy is necessary for surgical therapy	an earlier surgical procedure in the affected surgical field
	segmental resection or maxillectomy necessary for surgical therapy

3.3. Treatment strategies and study protocol

On the first visit, when a patient shows up at the Department with their complaints for the first time, the diagnosis is established based on a physical examination, the anamnestic and treatment parameters of the underlying disease and an orthopantomogram. The stage is also classified and during the physical examination, a sample is also taken from the pus or the exposed bone surface for microbiological examination. The patient is registered for a Cone Beam CT (CBCT) scan, a blood test, an ECG and a chest X-ray.

On the second visit, the CBCT scan is reviewed, and the size and localisation of the necrotic bone area are defined. Anaesthesia consultation is also held during which the general condition of the patient is ascertained based on the results of the blood test, the ECG and the chest X. Depending on the size and the localisation of the exposed bone area and the general condition of the patient, the treatment plan is set up. Depending on the treatment plan, the patient is registered for an operation and/or provided with an antibiotic prescription and medical advice.

The treatment strategy depends on the patient's general condition, the MRONJ stage and the size and the localisation of the exposed bone part. If the patient's general condition allows for surgical therapy and the patient consents to the operation, the treatment plan is surgical intervention. If the patient's general condition does not allow for surgical therapy or the patient doesn't consent to the operation, the treatment plan is conservative therapy. If surgical intervention is possible, surgical treatment is recommended in our Department in every case, because the chance for total recovery is higher, and the prognosis and the patient's quality of life are more favourable after surgical procedures than in the case of conservative treatment. Depending on the size and the localisation of the exposed bone part, local or general anaesthesia is used during the surgical intervention. For 3 days before and for 10 days following the procedure, an antibiotic is administered to the patient. The surgical intervention is performed with a minimally invasive approach, striving to remove all of the necrotic bone parts but taking out as little bone stock as possible. In stage 1 and stage 2, the surgical intervention means an alveolectomy or a marginal resection. In stage 3, we aim for preserving the continuity of the mandible in the case of lower jaw necrosis. If it is possible, the marginal resection is made with extraoral fistula excision. If the bilateral cortical of the lower jaw is not or only partially affected, the tunnel technique is used to remove the spongiosa and to maintain continuity.

Segmental resection is performed only if absolutely necessary. After segmental resection, immediate or delayed reconstruction is feasible with a fibula flap. In stage 3, partial maxillectomy is performed on the upper jaw. After the operation, local wound care, antimicrobial mouth rinses and pain control are performed in addition to the administration of antibiotics. The conservative treatment includes local wound care, antimicrobial mouth rinses, pain control and antibiotics application, initially in empirical form and later according to the antibiogram.

In case of surgical treatment, the patient is admitted to the inpatient care unit one day before the surgical intervention. The patient receives detailed information about the operation, its consequences and possibly complications, alternative treatment methods and about the expected consequences of failure to intervene. The patient is also fully informed about the study in which they are planned to be included. If the patient gives informed consent, the patient is included to the study. Anamnestic data, treatment parameters of the underlying disease and baseline characteristics of the MRONJ were recorded about each patient before the operation. If the patient is included in the first, genetic study, the genetic sample is taken from oral mucosa after receiving their informed consent. The genetic samples are collected at the Department and are delivered to the genetic laboratory in batches of 10-20 samples where the analysis is conducted. If the patient is included in the second, PRF study, the patient is randomly assigned to the experimental or the control group by random numbers generated by computer. If the patient is assigned to the experimental group, the surgical intervention is supplemented with A-PRF application. If the patient is assigned to the control group, a traditional operation is performed.

If conservative treatment is given, the patient can only be included in the first part of the genetic study, in which the role of SNPs in the development of MRONJ is examined. If the patient gives informed consent to the participation in the study, the genetic sample is taken at the first control examination. These genetic samples are collected and delivered with the other samples.

In the first study aimed at examining the role of SNPs in the development of MRONJ, the occurrence of SNPs is compared in the experimental group and the control group. The patients in the control group are also treated at the Department, but not for MRONJ. The genetic samples of these patients are taken after receiving their informed consent at the

inpatient or outpatient care unit. These genetic samples are collected and delivered with the other samples. The anamnestic data of the control patients are also recorded.

After surgical intervention, the patients are followed up. After the operation a control orthopantomogram is taken to record the bone status after the surgical treatment. The control examinations are at 1 week, 2 weeks, 1 month, 3 months, 6 months and 1 year after the operation or in case of complaints. At the control examinations, the state of the surgical site is recorded.

The data generated during the studies are recorded, aggregated and analysed. The results of the statistical analysis are evaluated and compared to the results of other publications.

3.4. Antibiotic treatment

If the necrosis of the jaw is in stage 2 and discharge is experienced at the first visit, empirical antibiotic administration is commenced. The first choice is amoxicillin/clavulanic acid (875/125 mg twice a day) (61). If the patient is allergic to penicillin, the first choice is levofloxacin (500 mg once a day). The duration of the antibiotic treatment is usually 2 weeks depending on the cessation of the discharge.

Oral antibiotic therapy is initiated 3 days before the operation and is continued for more than 10 days after the operation . The type of antibiotic administered depends on the results of the microbiological sampling at the first visit. The first choice is also amoxicillin/clavulanic acid (875/125 mg twice a day) (61). In case of resistance or the patient's penicillin allergy, the most frequently chosen agents are levofloxacin (500 mg once a day), moxifloxacin (400 mg once a day) or sulfamethoxazole and trimethoprim (400/80 mg, two tablets, twice a day).

If the postoperative histology confirms the presence of actinomyces species, the duration of the amoxicillin/clavulanic acid administration is extended to 4-6 weeks after surgical intervention. In patients allergic to penicillin, the type and duration of further antibiotic administration is based on the result of consultation with an infectologist.

3.5. Surgical therapy

Operations were performed under general or local anaesthesia, depending on the localisation and the extent of the bone defects. The surgical site was infiltrated with Lidocaine epinephrine 2% solution (61).

3.5.1. Alveolectomy, maxillectomy and marginal resection

Depending on the size and location of the necrotic bone part, an incision was made on the alveolar ridge that was extended beyond the edges of the necrotic parts (61). The alveolar ridge incision was supplemented with vertical incisions. The mucoperiosteal flap was created from these incisions to explore the necrotic bone area (61). After that, the affected bone area was completely removed up to the vital, bleeding bone surfaces (61). Depending on the extent and the localisation of the necrotic bone part, the affected areas were removed with a marginal resection, an alveolectomy or a partial maxillectomy (**Figure 1**). The sharp bone edges were rounded off. After the mucoperiosteal flap mobilisation, 1 mm was removed from the gingiva that was earlier around the denuded bone surface and the wound was closed with vertical mattress sutures (61).

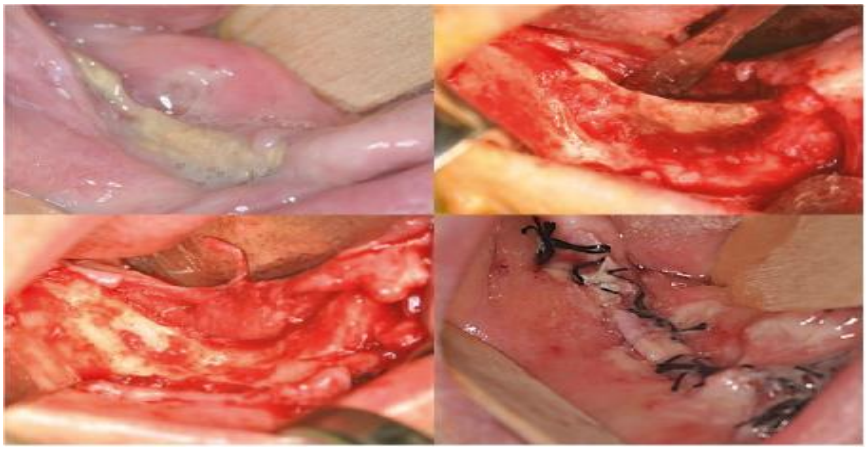


Figure 1: Steps of marginal resection (61)

3.5.2. Segmental resection

Depending on the presence of an intraoral or extraoral exposed bone part and the presence and size of an extraoral fistula, the surgical incision was made extraorally or intraorally. If an extraoral fistula is present, the fistula is circumcised and excised. From the extraoral or intraoral incision, the necrotic part of the lower jaw was approached and the edge of the necrotic bone part was explored. In the viable bone part next to the necrotic bone part, the exposed bone area was dissected from the viable bone part in the entire vertical height of the jawbone. The sharp bone edges were rounded off. After the flap mobilisation, 1 mm was removed from the gingiva/skin and the wound was closed in a double layer.

3.6. Preparation of the PRF membrane

In the second study, in the second patient group, the marginal resection or alveolectomy was supplemented with applying the A-PRF (**Figure 2**). The number of applied A-PRF membranes depended on the size of the bone defect. The A-PRF membranes were applied after the necrotic bone removal, in the site of the missing bone, to the bleeding bone surfaces (61). The flap mobilisation and the wound closure were the same as in the alveolectomy and marginal resection detailed above.

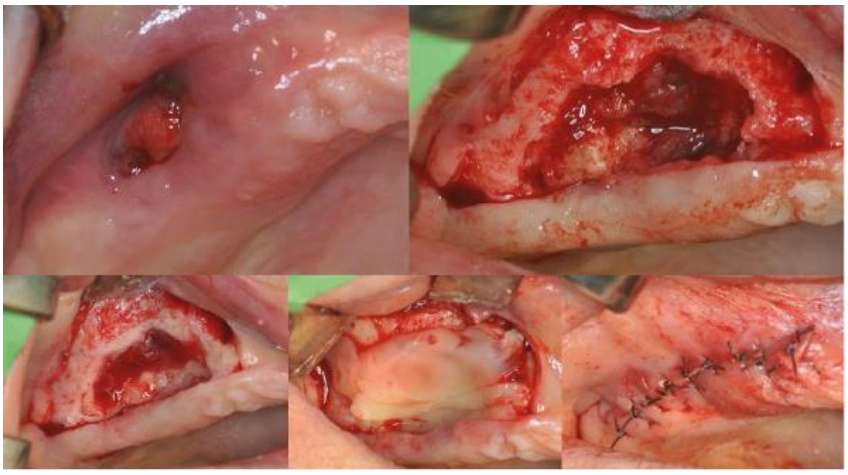


Figure 2: Steps of alveolectomy supplemented with A-PRF application (61)

The A-PRF membrane preparation followed the *Choukroun* method described in 2006 (95). The preparation was performed during the operation with the Choukroun PRF DUOTM Centrifuge System and the attached kit, according to the instructions. Depending on the size of the necrotic parts of the bone, 2 to 4 test tubes of peripheral venous blood were collected into 10 ml tubes of the kit (61). The tubes were placed immediately in the centrifuge and rotated at 1300 rpm for 14 minutes (61). The tubes were taken out of the Centrifuge and 5 minutes later the PRF was removed with surgical forceps from the tubes, separating the PRF layer from the acellular plasma layer above and the red blood cell layer below it (61). After that, the PRF was compressed to a membranous form for application (61).

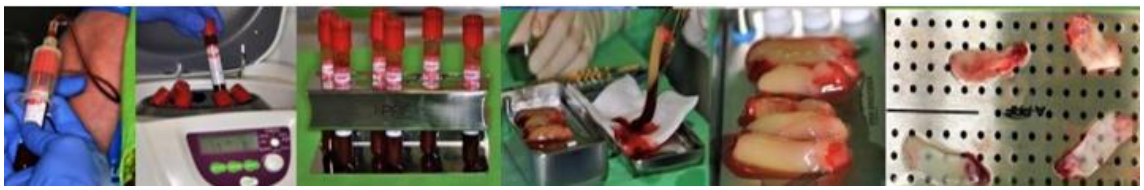


Figure 3: Steps of the PRF preparation (61)

3.7. Genetic examination

3.7.1. Genetic sampling

Cell scrapings from the oral mucosa were collected with the DentiGen Parodontitis Genetic Test (62). This test is appropriate for analysing the SNPs in position IL-1A-889 and IL-1B +3953 from intraoral cell scrapings (62).

3.7.2. Genetic analysis

The collected samples were analysed in the Istenhegyi Genetic Diagnostics Center (62). The first step of the analysis was DNA isolation after cell lysis on selective DNA binding. For DNA isolation, the KURABO QuickGene DNS kit was used. Then DNA was cleavage into segments with restricted endonucleases (62). The DNA segments were amplified with the PCR technique (One Taq Hot Start DNA polymerase) (62). DNA hybridization was used for the allelic analysis. The allelic analysis was performed with the Hain GenoType IL-1 kit. DNA samples were separated with agarose gel electrophoresis and then blotted onto a nitrocellulose membrane. The DNA was hybridized with dye-labelled probes (62).

3.7.3. Evaluation of genetic results

The genetic results show whether or not the patient carries SNP at the DNA position IL-1A-C889 or IL-1B+C3953. The test result also provides information on whether the patient has the defined SNP on all copies of the gene or only on one copy or none of the copies.

3.8. Follow-up

After surgical therapy, the follow-up period lasted for at least one year after the operation. The patients who did not participate in the control examinations or for whom the examination date was not registered were excluded from the studies. Control examinations were carried out at 1 week, 2 weeks, 1 month, 3 months, 6 months and 1 year after the operation or in case of complaints. The control examination contained a physical analysis and the precise registration of the condition of the surgical site based on the examination. Medical imaging was performed in all cases one day after the operation and when complaints or complications were suspected.

3.9. Outcome measure

In the follow-up period at control examinations, the surgical site was monitored and its condition was registered. The outcome of the disease was ascertained according to the condition of the surgical site, such as stage improvement, healing and recurrence.

Healing was registered when wound recovery with primary or secondary intention was observed and no signs of necrosis developed during the first 4 weeks after surgery. The presence of necrotic bone parts was probed with physical examination and medical imaging (61, 62).

Stage improvement was recorded when the disease reached a lower stage according to the 2014 recommendations of the AAOMS (8, 17, 61, 62).

Recurrence was recorded when the wound healed with primary or secondary intention, but after the recovery patients started experiencing complaints again or signs of necrotic bone parts were detected with physical examination or X-ray images (61, 62) .

In the first study, the recurrence number was an additional outcome. In case of recurrence, when the patients' conditions allowed for surgical therapy, repeated operations were performed. After the repeated operations, the surgical site was also monitored and the outcome parameters were registered including the recurrence. The number of recurrences was summarized during the follow-up period.

3.10. Data recording and statistical analysis

3.10.1. Data recording

Data were recorded anonymously based on patient code numbers. Anamnestic data, treatment parameters of the underlying disease and the baseline characteristics of the MRONJ were recorded about each patient before the operation. The genetic results were also entered into the table in the first study. The outcome parameters were also added to the table. Microsoft Excel 2013 was used for data recording.

3.10.2. Statistical analysis

Data were evaluated with StataBe18 (64-bit) and IBM SPSS Statistics 24. The significance level for statistical analysis was determined to be 0.05 (p=0.05).

In each study, the experimental group's collected data were compared to the comparison group's data to ensure the homogeneity of the groups and to exclude selection bias. The

variables of anamnestic, treatment and MRONJ baseline characteristics data are dichotomous, ordinal and continuous. Independent t-test, Wilcoxon rank sum test and Chi-squared test were used to compare the data.

3.10.2.1. Statistical analysis of the first study

In the first study, the variables of the genetic result were categorical. The stage improvement, the healing and the recurrence from the outcome parameters are dichotomous variables. The number of recurrences is a continuous variable. To examine the role of SNPs in the development of MRONJ *(Aim I/1)*, the occurrence of SNPs in the patient and the control group was compared with the Chi-squared test or Fischer exact test. To examine the role of SNPs in the prognosis of the disease *(Aim I/2)*, the occurrence of SNPs in patient groups with registered stage improvement or none, healing or none, recurrence or none was compared with the Chi-squared test or Fischer exact test. In the case of the recurrence number, the analysis was performed with an independent t-test or Wilcoxon rank sum test. The Chi-squared probe or Fischer exact test was applied to test the possible effect of SNPs on necrosis features *(Aim I/3)*. To examine the combined effect of SNPs with anamnestic and therapeutic data on the development and prognosis of MRONJ *(Aim I/4)*, model building, multivariable linear regression or multivariable logistic regression were used.

3.10.2.2. Statistical analysis of the second study

To test the effect of A-PRF in the prognosis of MRONJ, the stage improvement, healing and recurrence were compared in the experimental and comparison groups *(Aim II/1)*. The Chi-squared test or Fischer exact test was applied for the analysis. The effect of the necrosis features and the anamnestic and therapeutic data were also investigated in connection with the outcome of PRF-supplemented surgical therapy *(Aim II/2)*. For the analysis, multivariable logistic regression was applied.

4. Results

4.1. The results of the first, genetic study

In the first study, IL-1A and IL-1B defined SNPs' (IL-1A-889 C/T, IL-1B+3953 C/T) roles were examined in the development and prognosis of MRONJ. The SNPs' possible effect on necrosis features and their probable effect on the prognosis of MRONJ combined with anamnestic and therapeutic data were also investigated. In all five subsections of the study, the genetic results were analysed as described in the materials and methods section.

A total of 150 patients were included in the first study: 91 patients in the experimental group and 59 in the control group. The patients in the experimental group were diagnosed with MRONJ, while in the comparison group, no signs and symptoms of MRONJ could be detected. The collected data from the experimental and control groups are summarized in **Table 5**.

Table 5: The collected data (anamnestic and therapeutic, necrosis features) data in patients group and control group (62)

Anamnestic and therapeutic dates	Patient group	Control group
Numbers of the patients	91	59
Average age	69.82	70.22
Male: Female	37:54	18:41
Underlying disease		
Breast tumor	32 (35.16%)	0
Prostate tumor	25 (27.47%)	0
Multiple myeloma	14 (15.38%)	0
Osteoporosis	9 (9.89%)	0
Pulmonary tumor	6 (6.59%)	0
Renal tumor	5 (5.48%)	0
Diabetes mellitus	14 (15.38%)	16 (27.11%)
Chemotherapy	66 (72.52%)	0
Steroid therapy	34 (37.36%)	4 (6.77%)
Hormone therapy	48 (52.74%)	0
Bisphosphonate therapy	93 (100%)	0
Method of bisphosphonate administration		not applicable
intravenous or injection	65 (71.42%)	not applicable
per os	10 (10.98%)	not applicable
both	11 (12.08%)	not applicable
The type of bisphosphonate		not applicable
zoledronate	60 (65.93%)	not applicable
ibandronate	14 (15.38%)	not applicable
pamidronate	1 (1.09%)	not applicable
clodronate	2 (2.19%)	not applicable
aledronate	2 (2.19%)	
risedronate	4 (4.39%)	

Anamnestic and therapeutic dates	Patient group	Control group
Average time of bisphosphonate administration until	51.73	not applicable
Other antiresorptive drugs	22 (24.17%)	0
Smoking	23 (25.27%)	9 (15.25%)
Extractions before the development of the necrosis	72 (79.12%)	not applicable
Localization (jaw bone)		not applicable
Upper jaw	20 (21.97%)	not applicable
Lower jaw	56 (61.53%)	not applicable
Both	15 (16.48%)	not applicable
Localization (quadrant)		not applicable
1	4 (4.39%)	not applicable
2	10 (10.98%)	not applicable
3	25 (27.47%)	not applicable
4	15 (16.48%)	not applicable
Multiple quadrants	37 (40.65%)	
Localization (regio)		not applicable
Front region	7 (7.69%)	not applicable
Premolar region	14 (15.38%)	not applicable
Molar region	29 (31.86%)	not applicable
Multiple regions	41 (45.05%)	not applicable
Stage (at diagnosis)		not applicable
1	4 (4.39%)	not applicable
2	68 (74.72%)	not applicable
3	19 (20.87%)	not applicable

4.1.1. The examination of the defined SNPs' role in the development of MRONJ

In the experimental group 91, in the comparison group 59 patients were examined. The average age in the experimental group was 69.82 while it was 70.32 in the control group. In the patient group 37 men and 54 women, in the control group 18 men and 41 women were included. The experimental and the comparison group were homologous regarding age and gender. Genetic results were detectable from all samples both in the patient and in the control group. The genetic results of the experimental and control group are summarized in **Table 6**. The genetic results were evaluated as described in the materials and methods section.

Table 6: The genetic results of the experimental and control group (62)

	examined parameter	examination of the development		
		patient group	*control group*	*correlation with the development of the disease*
a,	the occurrence of the SNP on any copy of any of the two genes	51 (56.04%)	37 (62.71%)	p=0.418
b,	the occurrence of SNP in the given loci of any copi separately and together			
	in case of IL-1A gene	46 (50.54%)	31 (52.54%)	p=0.811
	in case of Il-1B gene	40 (43.95%)	30 (50.84%)	p=0.409

	examined parameter	examination of the development		
		patient group	*control group*	*correlation with the development of the disease*
	in case of Il-1A and IL-1B together	35 (38.46%)	24 (40.67%)	p=0.786
c,	the examined SNP is carried on all or one or none copies of the IL-1B gene			
	homozygous for wild-allelic variant (IL-1B+C3953, IL-1B+C3953)	51 (56.04%)	30 (50.84%)	p=0.778
	heterozygous for the SNP (IL-1B+C3953, IL-1B+3953T)	33 (36.26%)	23 (38.98%)	
	homozygous for the SNP (IL-1B+3953T, IL-1B+3953T)	7 (7.69%)	6 (10.16%)	
c,	the examined SNP is carried on all or one or none copies of the IL-1A gene			
	homozygous for the wild-allelic variant (IL-1A-C889, IL-1A-C889)	45 (49.45%)	29 (49.15%)	p=0.671
	heterozygous for SNP (IL-1A-889T, IL-1A-C889)	39 (42.85%)	23 (38.98%)	
	homozygous for the SNP (IL-1A-889T, IL-1A-889T)	7 (7.69%)	7 (11.86%)	
e,	in the given locus of IL-1A gene (IL-1A-889) and IL-1B gene (IL-1B+3953) on each copy what allelic variant is carried			
	IL-1A-C889, IL-1A-C889, IL-1B+C3953, IL-1B+C3953	39 (42.85%)	23 (38.98%)	p=0.859
	IL-1A-C889, IL-1A-C889, IL-1B+C3953, IL-1B+3953T	5 (5.49%)	5 (8.47%)	
	IL-1A-C889, IL-1A-C889, IL-1B+3953T, IL-1B+3953T	0	1 (1.69%)	
	IL-1A-889T, IL-1A-C889, IL-1B+C3953, IL-1B+C3953	10 (10.98%)	6 (10.16%)	
	IL-1A-889T, IL-1A-C889, IL-1B+C3953, IL-1B+3953T	27 (29.67%)	16 (27.11%)	
	IL-1A-889T, IL-1A-C889, IL-1B+3953T, IL-1B+3953T	3 (3.29%)	1 (16.94%)	
	IL-1A-889T, IL-1A-889T, IL-1B+C3953, IL-1B+C3953	0	0	
	IL-1A-889T, IL-1A-889T, IL-1B+C3953, IL-1B+3953T	2 (2.19%)	2 (3.38%)	
	IL-1A-889T, IL-1A-889T, IL-1B+3953T, IL-1B+3953T	5 (5.49%)	5 (8.47%)	

A, Out of the 150 examined patients, 88 carried at least one of the examined SNPs. The occurrence of the SNPs was 58.6%. In the experimental group 51 patients, in the control group 37 patients carried at least one of the examined SNPs on any copy of any of the two genes. No association was found between the carrying of SNPs and the development

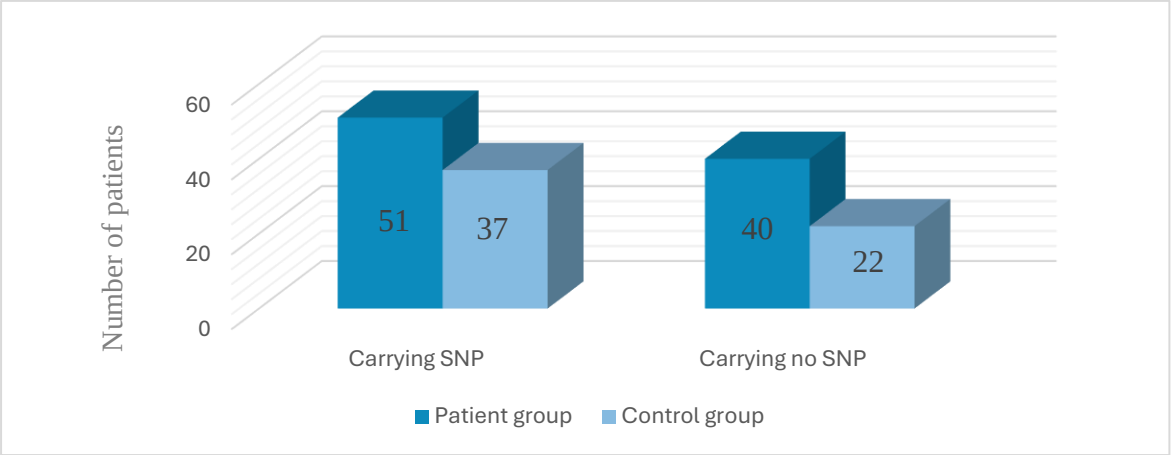


Figure 4: No association was found between the carrying of SNPs and the development of MRONJ (62)

of MRONJ (p=0.418). The result is shown in Figure 4 any of the two genes any of the two genes. No association was found between the carrying of SNPs.

B, The occurrence of the examined SNP was tested separately regarding IL-1A gene, IL -1B gene and both (IL-1A and IL-1B) genes. The occurrence of the IL-1A-889T SNP was 51.3% in the examined population. SNP (IL-1A-889T) was detected in 46 cases in the experimental group and in 31 cases in the control group on any copy of the IL-1A gene. No connection could be evinced between the presence of SNP on any copy of IL-1A and the development of necrosis (p=0.811).

The presence of IL-1B+3953T SNP was 46.6% in the studied population. IL-1B+3953T SNP was evincible in the patient group in 40 cases and in 30 cases in the control group. No correlation was shown between the development of MRONJ and the carrying of IL-1B+3953T SNP (p=0.409).

In the studied population, 59 patients, carried both IL-1A 889T and IL-1B+3953T SNPs. In the experimental group 35 and in the control group 24 cases were registered with both SNPs. No interrelation was found between the carrying of both SNPs and the development of necrosis (p=0.786).

C, The IL-1B+3953T SNP is presented on all, one or none of the copies of the IL-1B gene. 13 patients were homozygotic for the SNP, 56 (37.3%) heterozygotic for the SNP and 81 carried no IL-1B+3953T SNP. 7 of those homozygotic for the SNP were in the experimental group and 6 in the control group. Out of the 56 heterozygotic patients, 33 were in the experimental group and 23 in the comparison group. No connection was

detected between the development of the disease and the homozygotic or heterozygotic form of carrying the IL-1B+3953T SNP (p=0.778).

D, The IL-1A-889T SNP is presented on all, one or none of the copies of the IL-1A gene. On the basis of this, the patient could be homozygous or heterozygous for the SNP, and homozygous for the wild-type allele. 7-7 patients of the experimental and control group were homozygotic for the IL-1A-889T SNP, 39 in the experimental group and 23 patients in the comparison group carried SNP only on one copy. 74 patients - 45 out of the experimental and 29 of the control group - had only wild alleles. No connection was shown between MRONJ development and the homozygotic or heterozygotic form of carrying the IL-1A -889T SNP (p=0.671).

E, As described in the materials and methods section, 9 combinations of carrying the IL-1A-889T or IL-1B+3953T SNPs on one or two copies of the genes were also tested regarding the development of the disease. Out of the patients who carried SNP on any copy of the genes, the most frequent combination was being heterozygotic for IL-1A 889T and IL-1B+3953T SNPs. It occurred among 43 patients, out of which 27 were in the experimental and 16 in the comparison group. The second most frequent combination was being heterozygotic for IL-1A-889T and homozygotic for the wild allele of IL-1B. This combination was present in 16 cases: in 10 cases in the experimental and in 6 cases in the control group. The combinations and their occurrences are shown in **Table 5**. No association was detected between either of the combinations and the development of the disease (p=0.859).

4.1.2. The examination of the defined SNPs' role in the prognosis of MRONJ

The SNPs' role in the prognosis of MRONJ was examined in the experimental group among patients who were treated with surgical therapy. 77 patients were included in this section of the study. The collected data from the group are summarized in **Table 4**. The prognosis was evaluated on the basis of stage improvement, healing, recurrences, and number of recurrences. Out of the 77 patients, in 76 cases stage improvement, in one case no stage improvement, in 66 cases healing, in 8 (10.81%) cases no healing, in 31 cases recurrences, and in 34 cases no recurrences were detected. Out of the 33 patients who had recurrences, in 15 cases one recurrence, in 10 cases 2 recurrences and in 1 case 5 recurrences were discerned. The genetic results and the prognosis of the experimental

group are shown in **Table 7**. The genetic results were evaluated as described in the materials and methods section.

Table 7: The genetic results and the prognosis of the experimental group (62)

examined parameter	**examination of the prognosis**								
	stage improvement	*no stage improvement*	*correlation with the stage improvement*	*recovery*	*no recovery*	*correlation with the recovery*	*relapses*	*no relapses*	*correlation with the relapses*
a. the occurrence of the SNP on any copy of any of the two genes									
	48	0	p=0.195	40	5	p=0.917	24	15	**p=0.006**
b. the occurrence of SNP in the given loci of any copi separately and together									
case of the IL-1A gene	43	0	p=0.258	35	5	p=0.612	21	14	**p=0.032**
case of the IL-1B gene	37	0	p=0.308	31	5	p=0.407	22	11	**p=0.01**
case of IL-1A and IL-1B together	34	0	p=0.371	26	5	p=0.211	17	10	**p=0.034**
c. the examined SNP is carried on all or one or none copies of the IL-1B gene									
homozygous for the wild-allelic variant	37	1	p=0.595	35	3	p=0.396	11	23	**p=0.034**
heterozygous for the SNP	33	0		26	5		16	9	
homozygous for the SNP	6	0		5	1		4	2	
d. the examined SNP is carried on all or one or none copies of the IL-1A gene									
homozygous for the wild allelic variant	37	1	p=0.527	31	3	p=0.553	10	20	p=0.087
heterozygous for SNP	37	0		30	5		18	11	
homozygous for the SNP	6	1		6	0		3	3	
e. **in the given locus of IL-1A gene (IL-1A-889) and IL-1B gene (IL-1B+3953) on each copy what allelic variant is carried**									
IL-1A-C889. IL-1A-C889. IL-1B+C3953. IL-1B+C3953	28	1		25	4		6	19	
IL-1A-C889. IL-1A-C889. IL-1B+C3953. IL-1B+3953T	5	0		5	0		3	1	

examined parameter	examination of the prognosis								
	stage improvement	*no stage improvement*	*correlation with the stage improvement*	*recovery*	*no recovery*	*correlation with the recovery*	*relapses*	*no relapses*	*correlation with the relapses*
IL-1A-C889. IL-1A-C889 .IL-1B+3953T. IL-1B+3953T	0	0	p=0.939	0	0	p=0.578	0	0	p=0.128
IL-1A-889T. IL-1A-C889.IL-1B+C3953. IL-1B+C3953	8	0		8	0		4	3	
IL-1A-889T. IL-1A-C889.IL-1B+C3953. IL-1B+3953T	27	0		20	5		12	8	
IL-1A-889T. IL-1A-C889. IL-1B+3953T. IL-1B+3953T	3	0		3	0		2	1	
IL-1A-889T. IL-1A-889T. IL-1B+C3953. IL-1B+C3953	0	0		0	0		0	0	
IL-1A-889T. IL-1A-889T. IL-1B+C3953. IL-1B+3953T	2	0		2	0		1	1	
IL-1A-889T. IL-1A-889T. IL-1B+3953T. IL-1B+3953T	4	0		4	0		3	1	

A, Out of the 77 patients, 48 carried at least one of the examined SNPs. The occurrence of at least one of the examined SNPs was 62.33%. In the stage improvement group, 48 patients carried at least one of the examined SNPs on any copy of any of the two genes and 28 patients carried none of the examined SNPs. No patient carried SNP in the no stage-improvement group, and 1 patient who was detected with no stage-improvement, had a negative genetic result. No association was found between the carrying of the SNPs and the development of MRONJ (p=0.195). Among the 74 patients about whom data were recorded concerning their recovery, 45 carried at least one of the examined SNPs on any copy of any of the two genes. Out of the 45 patients, in 40 cases healing, and in 5 cases no healing was found. Out of the patients who had no examined SNP, in 26 cases recovery, in 3 cases no recovery was detected. No connection was evincible between

healing and the carrying of at least one of the examined SNPs (p=0.917). Out of the 65 patients about whom data were collected, 39 patients had at least one of the examined SNPs on any copy of any of the two genes. Out of the 39 patients, in 24 cases recurrence, while in 15 cases no recurrence was detected. Out of the patients with no SNP, in 7 cases recurrence, in 19 cases no recurrence was found. A significant association was detected between the carrying of at least one of the examined SNPs and recurrences (p=0.006). The result is shown on Figure 5. Out of the patients who had one recurrence, 12 patients carried either of the examined SNPs. Out of the patients with two recurrences, 8 patients had at least one of the examined SNPs. The patient with 5 recurrences also carried SNP. No connection was detected between the carrier of either of the SNPs and the number of recurrences (p=0.051).

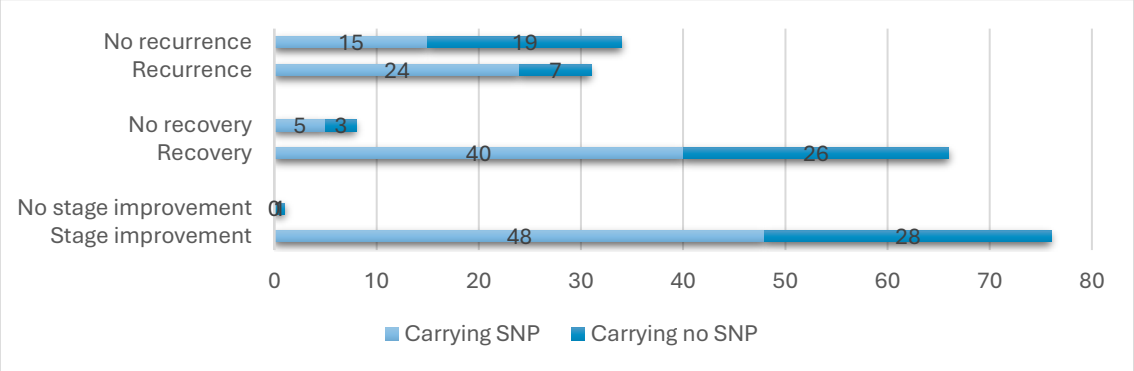


Figure 5: A significant association was detected between the carrying of at least one of the examined SNPs and recurrences (p=0.006) (62)

B. The occurrence of the IL-1A-889T SNP was 55.84% among surgically treated patients. SNP (IL-1A 889T) was detected in 43 cases. Out of the 43 cases all the cases showed stage improvement. Out of the patients who had no IL-1A-889T SNP, in 33 cases stage improvement was observed. One patient who had no stage improvement, carried no IL-1A-889T SNP. No relation was detected between stage improvement and the carrying of IL-1A-889T SNP (p=0.258). Out of the patients about whom data were recorded concerning recovery, 40 patients carried IL-1A-889T SNP. Out of these patients in 35 cases healing, in 5 cases no healing was found. Among the patients who had no IL-1A 889T SNP, in 31 cases healing, in 3 cases no healing was detected. No connection was found between the occurrence of IL-1A-889T SNP and the healing of MRONJ lesion (p=0.612). Out of the 65 patients about whom data were noted down concerning recurrences, 36 patients carried IL-1A-889T SNP. Among these patients in 21 cases

recurrences, in 14 cases no recurrences were observed. Among the patients who had no IL-1A-889T SNP, in 10 cases recurrence, in 20 cases no recurrence was noted. A significant association was found between the occurrence of IL-1A 889T SNP and recurrences (p=0.032). Out of the patients who had 1 recurrence, 11 patients carried IL-1A 889T SNP. Out of the patients with 2 recurrences, 6 patients had IL-1A 889T SNP. The patient with 5 recurrences also carried IL-1A 889T SNP. No connection was detected between the IL-1A 889T SNP and the number of recurrences (p=0.169).

The presence of IL-1B+3953T SNP was detected in 50,64% among patients who received surgical therapy. Among these patients, stage improvement developed in all cases. Among the patients who had no IL-1B+3953T SNP, stage improvement was found in 37 cases. One patient, who had no stage improvement, carried no IL-1B+3953T SNP. No relation was found between stage improvement and the carrying of IL-1B+3953T SNP (p=0.308). Out of the patients about whom data were collected concerning recovery, 36 patients had IL-1B+3953T SNP. Out of these patients in 31 cases healing, in 5 cases no healing was detected. Among patients who had no IL-1B+3953T SNP, in 35 cases healing, in 3 cases no healing was noted down. No connection was found between the occurrence of IL-1B+3953T SNP and surgical site healing (p=0.407). Out of the 65 patients about whom data were collected concerning recurrences, 31 patients carried IL-1B+3953T SNP. Among these patients, in 20 cases recurrences, and in 11 cases no recurrences were detected. Out of the patients who had no IL-1B+3953T SNP, in 11 cases recurrence, in 23 cases no recurrence was found. A significant association was revealed between the occurrence of IL-1B+3953T SNP and recurrences (p=0.010). Out of the patients who had one recurrence, 12 patients carried IL-1B+3953T SNP. Out of the patients with 2 recurrences, 5 patients had IL-1B+3953T SNP. The patient with 5 recurrences did not carry IL-1B+3953T SNP. A correlation was detected between the carrier of IL-1B+3953T SNP and the number of recurrences (p=0.019).

Among the patients who were treated with surgical therapy, 34 patients, carried both IL-1A-889T and IL-1B+3953T SNPs. Among these patients, stage improvement occurred in all cases. Out of the patients who did not have both IL-1A-889T and IL- 1B+3953T SNP, 42 patients showed stage improvement. 1 patient who had no stage improvement carried no SNP. No relation was revealed between stage improvement and the carrying of both IL-1A-889T and IL-1B+3953T SNPs (p=0.371). Out of the patients about whom data

were recorded concerning recovery, 31 patients had IL-1A-889T and IL-1B+3953T SNPs. Among these patients, in 26 cases healing, and in 5 cases no healing was found. Among the patients who did not have both SNPs, in 40 cases healing, in 3 cases no healing was detected. No correlation was found between the occurrence of both SNPs and wound healing (p=0.211). Out of the 65 patients about whom data were collected concerning recurrences, 27 patients carried both SNPs. Out of these patients, in 17 cases recurrences, and in 10 cases no recurrences were noted down. Among the patients who did not have both SNPs, in 14 cases recurrence, in 24 cases no recurrence was recorded. A significant association was found between the occurrence of both IL-1A 889 T and IL-1B+3953T SNPs and recurrences (p=0.034). Out of the patients who had one recurrence, 11 patients carried both SNPs. who had two recurrences, 3 patients had both SNPs. The patient with 5 recurrences did not carry both IL-1A-889T and IL-1B+3953T SNPs. An association was detected between the carrier of both IL-1A 889T and IL-1B+3953T SNPs and the number of recurrences (p=0.026). The results are shown in **Figure 6**.

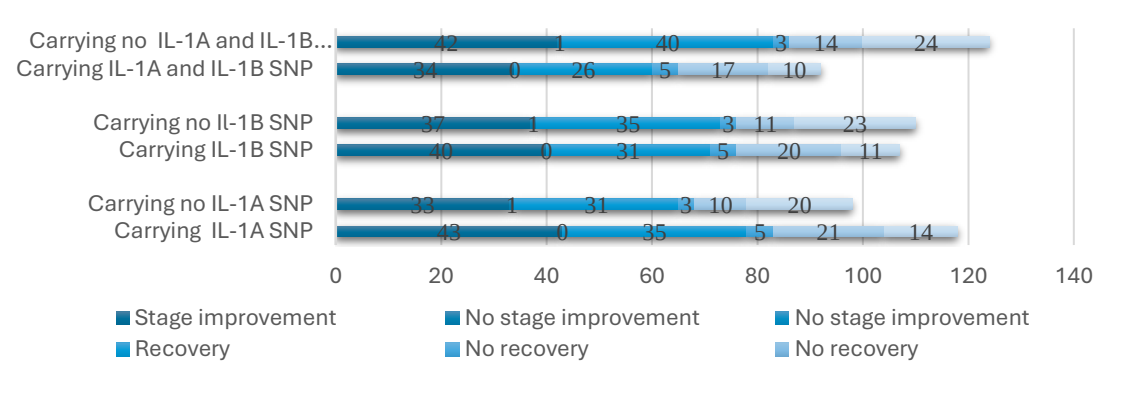


Figure 6: A significant association was detected between the separately carrying of the examined SNPs and recurrences (p=0.06) (62)

C, Out of the patients who were surgically treated 6 patients were homozygotic for the IL-1B+3953T SNP, 33 heterozygotic for the IL-1B+3953T SNP and 38 carried no IL-1B+3953T SNP. Out of the 6 patients who carried IL-1B+3953T SNP on all copies of the IL-1B gene, 6 patients had stage improvement and no patients were detected with no stage improvement. Out of 33 patients who carried IL-1B+3953T SNP on one of the copies of the IL-1B gene, stage improvement was found among all 33 patients. Out of the 38 patients who carried IL-1B+3953T SNP on none of the copies of the IL-1B gene, 37 patients were detected with stage improvement and in one case no stage improvement was discerned. No association was found between the carrying of the IL-1B+3953T SNP

on all, one or none of the copies of the IL-1B gene and stage improvement (p=0.595). Out of the 6 patients who were homozygotic for the IL-1B+3953T SNP, in 5 cases healing, in one case no healing was observed. Among the patients heterozygotic for the IL-1B+3953T SNP, in 26 cases healing, in 5 cases no healing was detected. Out of the patients who carried no IL-1B+3953T SNP on none of the copies of the IL-1B gene, in 35 cases healing, in 3 cases no healing was found. No connection was detected between the carrying of the IL-1B+3953T SNP on all, one or none of the copies of the IL-1B gene and healing (p=0.396). Out of the patients who were homozygotic for the IL-1B+3953T SNP, in 4 cases recurrence, in 2 cases no recurrence was discerned. Out of the patients who were heterozygotic for the IL-1B+3953T SNP, in 16 cases recurrence, in 9 cases no recurrence was noticed. Out of the patients who carried no IL-1B+3953T SNP on none of the copies of the IL-1B gene, in 11 cases recurrence, and in 23 cases no recurrence was found. A significant association was detected between the carrying of the IL-1B+3953T SNP on all, one or none of the copies of the IL-1B gene and recurrence (p=0.034). The result is shown on **Figure 7**. Out of the patients who were homozygotic for the IL-1B+3953T SNP, one patient had 1 recurrence and one patient had 2 recurrences. Among the patients who were heterozygotic for the IL-1B+3953T SNP, in 10 cases 1 recurrence, in 4 cases 2 recurrences were observed. Out of the patients who carried no IL-1B+3953T SNP on none of the copies of the IL-1B gene, in 3 cases 1 recurrence, in 5 cases 2 recurrences and in 1 case 5 recurrences were noticed. No connection was detected between the carrying of the IL-1B+3953T SNP on all, one or none of the copies of the IL-1B gene and the recurrence number (p=0.126).

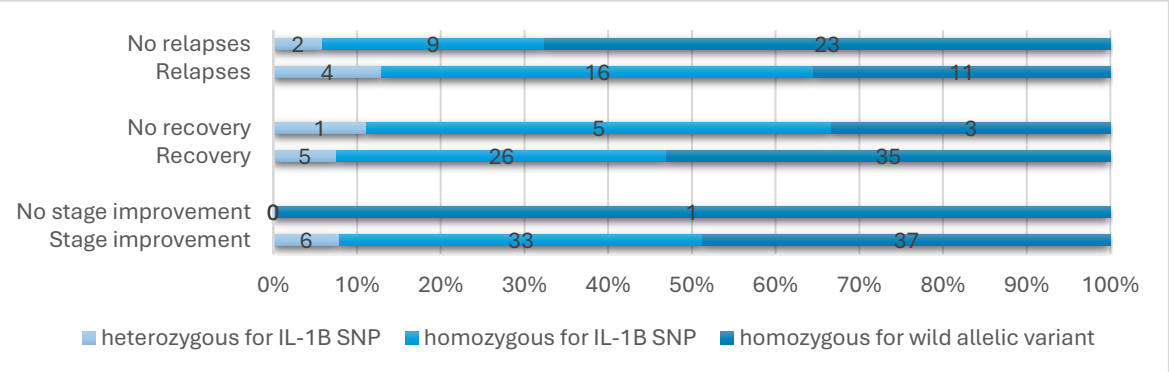


Figure 7.: A significant association was detected between the carrying of the IL-1B+3953 T SNP on all, one or none of the copies of the IL-1B gene and recurrence (p=0.034) (62)

D, Out of the patients who were surgically treated 6 patients were homozygotic for the IL-1A-889T SNP, 37 heterozygotic for the IL-1A-889T SNP and 34 carried no IL-1A 889 T SNP. Out of the 6 patients who carried IL-1A 889 T SNP on all copies of the IL-1A gene, 6 patients were detected with stage improvement and no patients were detected with no stage improvement. Out of 37 patients who carried IL-1A 889T SNP on one of the copies of the IL-1A gene, stage improvement was noticed in all cases. Out of the 38 patients who carried IL-1A-889T SNP on none of the copies of the IL-1A gene, 37 patients were detected with stage improvement, while in 1 case no stage improvement was found. No association was detected between the carrying of the IL-1A-889 T SNP on all, one or none of the copies of the IL-1A gene and stage improvement ($p=0.527$). Out of the 6 patients who were homozygotic for the IL-1A-889 T SNP, in all cases healing was observed. Among the heterozygotic patients for the IL-1A-889T SNP, in 30 cases healing, in 5 cases no healing was observed. Out of the patients who carried no IL-1A-889T SNP on none of the copies of the IL-1A gene, in 31 cases healing, in 3 cases no healing was found. No connection was detected between the carrying of the IL-1A-889 T SNP on all, one or none of the copies of the IL-1A gene and healing ($p=0.553$). Out of the patients who were homozygotic for the IL-1A 889T, in 3 cases recurrence, in 3 cases no recurrence was discerned. Among the patients who were homozygotic for the IL-1A-889 T SNP, in 18 cases recurrence, in 11 cases no recurrence was noticed. Out of the patients who carried no IL-1A-889T SNP on none of the copies of the IL-1A gene, in 10 cases recurrence, and in 20 cases no recurrence was found. No association was detected between the carrying of the IL-1A-889 T SNP on all, one or none of the copies of the IL-1A gene and recurrence ($p=0.087$). Out of the patients who were homozygotic for the IL-1A-889T SNP, 2 patients had 1 recurrence. Out of the patients who were heterozygotic for the IL-1A-889T SNP, in 9 cases 1 recurrence, in 6 cases 2 recurrences, and in 1 case 5 recurrences were noticed. Out of the patients who carried no IL-1A-889T SNP on none of the copies of the IL-1A gene, in 4 cases 1 recurrence, in 4 cases 2 recurrences were found. No connection was detected between the carrying of the IL-1A 889T SNP on all, one or none of the copies of the IL-1A gene and recurrences ($p=0.324$).

E, As described in the materials and methods section, 9 combinations of carrying the IL-1A-889T or IL-1B+3953T SNPs on one or two copies of the genes were also tested regarding the prognosis of the disease. Out of the patients who were treated with surgical

therapy and carried SNP on any copy of the genes, only 7 combinations were found and the most frequent combination was being heterozygotic for IL-1A-889T and IL-1B+3953T SNPs, which occurred among 27 patients. Among the 27 patients, in all cases stage improvement, in 20 cases healing, in 5 cases no healing, in 12 cases recurrences and in 8 cases no recurrences were detected. Among the 27 patients, in 8 cases 1 recurrence, and in 2 cases 2 recurrences were noticed. The second most frequent combination was being heterozygotic for IL-1A-889T and homozygotic for the wild allele of IL-1B. This combination was presented in 8 cases. Among the 8 patients, in all cases stage improvement, in all cases healing, in 4 cases recurrences and in 3 cases no recurrences were detected. Among the 8 patients, in 3 cases 2 recurrences, and in 1 case 5 recurrences were noticed. The other 4 combinations that were detected in this group: homozygosity for both IL-1A-889T and IL-1B+3953T on all of the copies of the two genes (4 cases,); in the case of IL-1A gene, heterozygosity for IL-1A 889T and in the case of IL-1B gene, homozygosity for IL-1B+3953T (3 cases); in the case of IL-1A on all copies, it carried wild allele and in the case of IL-1B, heterozygosity for IL-1B+3953T (5 cases,); in the case of IL-1A on all copies it carried IL-1A- 889T and in the case of IL-1B gene, heterozygosity for IL-1B+3953T (2 cases). In the 4 combinations, stage improvement and healing were detected in all cases. In the case of the combination of homozygosity for both IL-1A-889T and IL-1B+3953T on all of the copies of the two genes, in 3 cases recurrences and in 1 case no recurrences were detected. In the case of the combination with IL-1A gene being heterozygotic for IL-1A-889T and in the case of IL-1B gene, being homozygotic for IL-1B+3953T, in 2 cases recurrences, in 1 case no recurrence was noticed. In the combination with IL-1A on all copies carrying wild allele and in the case of IL-1B with heterozygosity for IL-1B+3953T, in 3 cases recurrences and in 1 case no recurrence was found. In the case of the combination of IL-1A on all copies carrying IL-1A-889T and in the case of IL-1B gene with heterozygosity for IL-1B+3953T, in 1 case recurrence and in 1 case no recurrence was detected. The number of recurrences in the cases of the 4 combinations is also shown in **Table 6**. No association was detected between the combinations and stage improvement (p=0.939), healing (p=0.578), recurrences (p=0.128) and the number of recurrences (p=0.49).

4.1.3. Examination of the defined SNP's effect on the necrosis features

In this part of the study, the IL-1A-889T and IL-1B+3953T SNP's effect on the necrosis features was examined in the experimental group, among patients who suffered from MRONJ. The examined features were the stage at the time of the diagnosis, the localisation of the affected bone area, and whether tooth extraction triggered the development of the necrosis. The affected bone localisation was analysed according to jaw, jawbone region and quadrant. The data about these features are included in **Table 7**. The genetic results were evaluated as described in the materials and methods section.
The results are shown in **Table 7**. Any of the defined SNPs' affect the necrosis features. Only the most frequent combination (heterozygotic for IL-1A 889T and IL-1B+3953T SNPs) is shown in **Table 7**. from the 9 combinations, due to the low case number and through them the statistically irrelevant result. In several steps in the analysis, statistically irrelevant result is detected due to the low case number in subgroups. These statistical results are shown in parenthesis in **Table 8**.

Table 8: Examination of the defined SNPs' effect on the necrosis features (62)

examined parameter	**examination of the necrosis features** (number of patients)											
	Stage				**Localisation (jaw)**			**Localisation (regio)**			**Extraction**	
	stage 1	*stage 2*	*stage 3*	*correlation (stage)*	*upper jaw*	*lower jaw*	*correlation (jaw)*	*front region*	*premolar/molar*	*correlation (region)*	*extraction*	*correlation (extraction)*
a, the occurrence of the SNP on any copy of any of the two genes												
	4	39	7	p=0.67	11	34	p=0.34	12	36	p=0.57	42	p=0.65
b, the occurrence of SNP in the given loci of any copi separately and together												
case of the IL-1A gene	4	34	7	p=0.80	9	31	p=0.46	10	33	p=0.75	38	p=0.82
case of the IL-1B gene	2	32	6	p=0.53	7	31	p=0.2	12	26	p=0.18	33	0.88
case of IL-1A and IL-1B together	1	27	6	p=0.78	5	28	p=0.20	10	23	p=0.34	29	p=0.89

examined parameter	examination of the necrosis features (number of patients)											
	Stage				Localisation (jaw)			Localisation (regio)			Extraction	
	stage 1	*stage 2*	*stage 3*	*correlation (stage)*	*upper jaw*	*lower jaw*	*correlation (jaw)*	*front region*	*premolar/molar*	*correlation (region)*	*extraction*	*correlation (extraction)*
c, the examined SNP is carried on all or one or none copies of the IL-1B gene												
heterozygous for the SNP	2	25	6	p=0.54	7	24	p=0.43	9	22	p=0.31	29	p=0.61
homozygous for the SNP	0	7	0		0	7		3	4		4	
d, the examined SNP is carried on all or one or none copies of the IL-1A gene												
heterozygous for SNP	4	29	5	p=0.13	9	25	p=0.48	7	31	p=0.24	34	p=0.84
homozygous for the SNP	0	5	2		0	6		3	3		4	
e, in the given locus of IL-1A gene (IL-1A-889) and IL-1B gene (IL-1B+3953) on each copy what allelic variant is carried												
IL-1A-889T, IL-1A-C889, IL1B+C3953, IL-1B+3953T	2	20	5	p=0.02	5	20	p=0.39	7	19	p=0.21	24	p=0.004

4.1.4. Examination of the SNP's probable effect on the prognosis of MRONJ combined with anamnestic and therapeutic data

The SNP's probable effect combined with anamnestic and therapeutic data on the prognosis of MRONJ were tested with logistic regression. No necrosis features or the anamnestic or therapeutic data were found which combined with SNPs affect the prognosis of the disease.

4.2. The results of the second, PRF study

The second study tested the effectiveness of surgical therapy supplemented with the application of platelet-rich fibrin membrane on the prognosis of the disease. The effect of

A-PRF usage on the prognosis of MRONJ was examined by comparing the prognostic features, such as stage improvement, healing, type of healing, recurrences and the number of recurrences in the experimental and control groups. In the comparison group, the patients were treated with traditional surgical therapy. In the experimental group, traditional surgical treatment was supplemented with A-PRF membrane application. The study also investigated the effect of the necrosis features and the anamnestic and therapeutic data on the outcome of the PRF-supplemented surgical therapy.

4.2.1. Experimental group and comparison group

82 patients diagnosed with MRONJ and treated with surgical therapy were included in the study. 38 patients were randomized to the experimental group and 44 patients to the comparison group. In both anamnestic and therapeutic patient data were registered. The parameters examined in the study are indicated in **Table 8**. There were no statistically detectable differences in the registered parameters within the groups (61). Both groups were homologous in terms of the parameters listed in **Table 9** (61).

Table 9: Anamnestic and therapeutic data of experimental and control group

Anamnestic and therapeutic dates	Patient group	Control group	Difference
Number of the patients	38	42	
Average age	69.96	71.38	p=0.913
Male: Female	15:23	10:34	p=0.080
Underlying disease			
Breast tumor	14	23	p=0.168
Prostate tumor	11	6	
Multiple myeloma	5	10	
Osteoporosis	4	2	
Pulmonary tumor	2	0	
Other tumors	3	2	
Diabetes mellitus	8	8	p=0.479
Chemotherapy	24	31	p=0.215
Steroid therapy	15	18	p=0.543
Hormone therapy	24	24	p=0.226
Method of bisphosphonate administration			p=0.061
intravenous or injection	26	36	
per os	3	0	
both	2	7	
The type of bisphosphonate			
zoledronate	18	24	p=0.279
ibandronate	8	10	
clodronate	0	1	

Anamnestic and therapeutic dates	Patient group	Control group	Difference
aledronate	1	0	
risedronate	1	0	
Other antiresorptive drugs	14	13	p=0.320
Smoking	6	11	0.229
Extractions before the development of the necrosis	25	22	p=0.100
Localization (jawbone)			p=0.551
Upper jaw	16	15	
Lower jaw	19	27	
Both	3	2	
Localization (quadrant)			p=0.811
1	11	11	
2	3	6	
3	8	12	
4	7	11	
Multiple quadrants		42	
Localization (regio)			0.073
Front -premolar region	20	14	
Premolar-molar region	18	30	
Stage (at diagnosis)			0.442
1	2	1	
2	31	33	
3	5	10	

4.2.2. Examination of the A-PRF usage effect on the prognosis of MRONJ

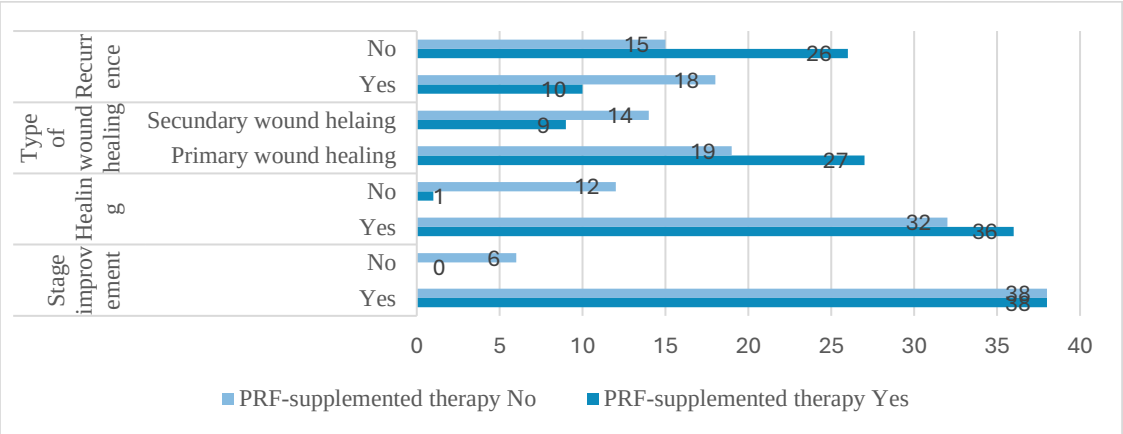
Stage improvement was detected in 38 cases (100%) in the experimental group and in 38 (86.36%) cases in the comparison group following the surgical therapy. Regarding the stage improvement, the experimental group's results were significantly better than the comparison group's outcomes (Fisher's Exact Test, p=0,020) (**Figure 8**).

Healing was found in 68 cases (83.95%), in the experimental group 36 (97.29%) cases and in the comparison group 32 (72.72%) cases. Insufficient wound healing was detected in the experimental group in 1 case (2.7%) and in the control group 12 (27.27%) cases. Primary wound healing was seen in the experimental group in 27 cases (75.00%) and in the control group in 19 cases (57.57%). Secondary wound healing was discerned in the experimental group in 9 cases (25%) and in the comparison group in 14 cases (42.42%). A significant difference was found between wound healing of the comparison group and the experimental group (Fisher Exact Test, p=0.002). The results of healing in both groups are shown in **Figure 8**.

In the experimental group, primary or secondary intention wound healing was detected in 36 patients, out of whom in 10 (27.77%) cases recurrences were found. In this group, no relapses were developed in the follow-up period in 26 (72.22%) cases. In the comparison

group, relapses were discerned in 18 (54.54%) cases after wound healing. No relapses were found in this group during the follow-up period in 15 (45.45%) cases. Out of patients who experienced relapses, in the experimental group 1-3 relapses, in the comparison group 1-5 relapses were detected. Significantly fewer relapses were observed in significantly fewer cases in the experimental group than in the comparison group (Fisher Exact Test, p=0.022) The difference in relapses between the two groups is shown in **Figure 8.**

Figure 8.: The effect of PRF-supplemented therapy to the prognosis of MRONJ



In the experimental group, among patients treated with A-PRF-supplemented surgical therapy, significantly better results were detected in terms of stage improvement and healing, and significantly fewer relapses were found than in the comparison group.

4.2.3. Examination of the effect of the necrosis features, the anamnestic and therapeutic data on the outcome of the PRF-supplemented surgical therapy

The necrosis features, the anamnestic and therapeutic data on the outcome of the PRF-supplemented surgical therapy were tested with logistic regression. No necrosis features or the anamnestic or therapeutic data were found which affect the outcome of the PRF-supplemented surgical therapy.

5. Discussion

Although MRONJ develops in only 0.01-19% of patients treated with antiresorptive therapy, the prognosis of the disease is so unfavourable and the disease causes such a decrease in the quality of life of patients that clarifying the factors that play a role in the development and prognosis of the disease is important (1, 113). Clarifying these factors may help develop more elaborate preventive and treatment strategies, open the possibility of working out and applying new and more effective treatment options, and help identify details in the pathomechanisms of MRONJ (41). Since the first description of the disease, several factors have been identified that are associated with the development and prognosis of MRONJ. Based on publications, some factors seem to be related to the development of the disease, such as the underlying disease, the type and duration of antiresorptive therapy, anatomic factors and so on (119-121). Factors that show an association with the prognosis of the disease according to publications among others are the treatment type, multiple myeloma as the underlying disease, the staging of MRONJ (59, 68, 122, 123).

In the last 10-15 years, the possibility that genetic factors play a role in the development and the pathomechanism of the disease has been considered. Several studies were published that investigated the probable genetic factors in the development of MRONJ. The AAOMS recommendation in 2022 highlighted the investigation of the SIRT1 gene's SNPs in MRONJ (1). In this investigation, *Yang et al.* found that the upregulation of the SIRT1 gene due to SNPs in the promoter region led to a lower presence of MRONJ (124). Several other genes' SNPs have also shown an association with the development of MRONJ in publications, such as ESR1, PPAR gamma, *RBMS3*, CYP2C8, COL1A, RANK, MMP2, OPG, OPN，MHC II, FDPS and VEGF (125-128). Some of these SNP's role in the development of MRONJ is controversial. *Guo et al.* found no association between CYP2C8 and PPARG and the development of MRONJ (129). However, *English et al.*, *Balla et al.* and *Such at al.* detected an association between CYP2C8 and the risk of MRONJ (130-132). *Arduino et al.* couldn't identify the connection between the development of MRONJ and the same VEGF's SNPs that *Choi et al.* investigated in 2015 (133, 134). *Yang et al.* and *Nicoletti et al.* in their genome-wide association studies also detected a connection between the development of the disease and rs2736308 on chromosome 8 and RBMS3 gene's defined SNP (124, 126). Only a few whole genome

and whole exome studies have been published that examined the genetic background of MRONJ. In one of them, *Yang et al.* found that SIRT1/HERC4 Locus SNPs are associated with BRONJ (108). Until now, only a few studies have been published that examine the potential role of genetic factors in the prognosis of MRONJ. *Kim et al.* identified an association between KRT18 and PABPC3 genes' nine SNPs and MRONJ recurrences in their whole exome sequencing study on 10 patients (135). *Bojtor et al.* found a significant association between SIRT1's SNP and the stage improvement after surgical therapy in MRONJ (20).

No study has been published on examining Interleukins' genetic variants, including Interleukin-1 SNPs, in the development and prognosis of MRONJ. The hypothesis of this study was based on the one hand on IL-1's regulatory role in bone remodeling, immune response and the inflammatory process, which are presumed to be factors in the pathomechanisms of MRONJ. On the other hand, the SNPs of the IL-1A and IL-1B genes have been described in several autoimmune and inflammatory diseases, such as rheumatoid arthritis and chronic periodontitis (136-138).

The studied population in this examination according to demographic factors, such as age and gender, corresponds with the other studies that investigated the genetic predisposition of MRONJ (41). Some studies described a gender ratio similar to this examination (139, 140). In respect of the underlying disease, the data recorded in this study do not correspond to the results of other genetic investigations. Based on the data aggregated by da *Silva et al.*, in genetic predisposition examinations, the most common underlying disease is multiple myeloma (50.4%) (41). The second and third most common are prostate cancer (17.4%) and breast cancer (17.9%) (41). The most common underlying diseases are breast cancer (29.5%), prostate cancer (29.5%) and osteoporosis (17.9%) (41). Multiple myeloma is detected only in 9% of the patients (41). The distribution of the underlying diseases is diverse in the publications depending on the studied population. Based on multicentric studies and systematic reviews, solid tumors in approximately 50-60%, multiple myeloma in approximately 20% and osteoporosis in approximately 15% are described as the underlying disease in patients' anamneses (139, 141). Some studies described a proportion of patients suffering from multiple myeloma similar to this study (142). Diabetes mellitus is registered in 14.1% of the patients, which is similar to the ratios recorded in other studies published on MRONJ (141-143). All

patients received bisphosphonate therapy, and in 26.3% of the patients, the bisphosphonate therapy was changed to denosumab after a certain treatment time. In most published studies, the proportion of zoledronate and denosumab administration is the same as in this study (139). The most administered BPs were zoledronate (71.6%) and ibandronate (11.4%). Other BPs, such as pamidronate, alendronate, risedronate and clodronate, were used only in 1-3% of the patients. The most frequently administered bisphosphonate in publications is zoledronate, which is used in 60-70% of the cases (144). The administration ratio of other BPs is varied in publications, used as follows: pamidronate in approximately 7-14%, ibandronate in approximately 3.7-6%, alendronate in approximately 9%, and risedronate in approximately 3% (139, 144). Based on the data aggregated by *da Silva et al.*, in genetic predisposition examinations, the most administered BP was zoledronate (68.8%) and the proportion of other BPs administered was like those published in other studies (41). Antiresorptive therapy was administered intravenously or in injections in 84.6% and orally in 11.5% of patients. The average administration time of BPs until the occurrence of the disease was 44.7 months. The data in this studied population on the administration form and time of BPs are matched with data recorded in other publications concerning MRONJ (143, 145). 52.6% of the patients received hormonal/endocrine therapy, 37.2% were treated with steroids and 70.5% of the patients received chemotherapy in connection with the treatment of the underlying disease. The data on hormonal, steroid and chemotherapy published in other papers are compared with the data recorded in this study (139). Tooth extraction or oral surgery procedures preceded the occurrence of MRONJ in 82.1% of the cases. Tooth extractions are registered in a lower percentage of cases before the occurrence of MRONJ in the publications, in approximately 50% of the patients (139, 143, 144). The patients were diagnosed with MRONJ in stage 1 in 3.8%, in stage 2 in 78.2% and in stage 3 in 17.9%. The published studies reported diverse proportions of the disease stage at diagnosis. In several studies, the proportion of patients diagnosed with stage 1 is higher and stage 2 is lower than in this studied population (139, 141). Some studies reported stage ratios similar to this study (144). The surgical site was healed in 88.6% of the cases and recurrence was detected in 41.7% of the patients who healed after the operation. The surgical site is healed in 53.4%-84.6% of the cases in the investigations published on MRONJ (54). The proportion of healing after surgical therapy in this studied population is slightly higher

than the published. In publications, the recurrence rate is between 11.8% and 45% (55, 59, 146). The proportion of recurrences in this study corresponds to the data in the literature.

The defined IL-1A and IL-1B polymorphisms occur in 58.66% of the patients. In the experimental group, the presence of SNPs is 56.04%, and in the comparison group it is 72.54%. IL-1A-889 T was detected in 46 (50.54%) cases in the experimental group and in 31 (52.54%) cases in the control group. The presence of IL-1B+3953T SNP is 46.6% in the studied population. IL-1B+3953T SNP was evincible in the patient group in 40 (43.95%) cases and in the control group in 30 (50.84%) cases. Although no study has been published on IL-1 polymorphisms in osteonecrosis, several studies have examined IL-1 SNPs in other diseases. Some of these studies tested the occurrence of IL-1 polymorphisms in an experimental group compared to a control group, so these publications provided data about the presence of SNPs in the healthy population. *Brodzikowska et al.* examined IL-1A−889 and IL-1B+3953 SNPs in the Polish population concerning periodontitis. In the comparison group, which was comprised of healthy people, the presence of IL-1A-889T was 40%, and the occurrence of IL-1B3954T was 30% (147). *Cardoso et al.* investigated the defined SNPs concerning peri-implantitis in a Portuguese population. In the control group, the presence of the IL-1A-889 SNP was 15% and IL-1B+3953 was 10% (148). *Wagner et al.* tested patients with chronic periodontitis compared to the control group in the Caucasian population. In the comparison group, the patients carried IL-1A-889T in 53% and IL-1B+3953 in 43% (149). *Zhang et al.* published a meta-analysis in 2010, which investigated several polymorphisms in Interleukin-1 concerning sepsis in Caucasian and Asian populations based on the aggregation of the results of several publications (86). In *Zhang et al.*'s meta-analysis, which was based on Davis et al.'s publication, IL-1A-889T was present in the control group in 53.84% in the Caucasian population (86) (150). In *Johnson et al.*'s publication IL-1B+3953T was present in the control group in 39.92% in the Caucasian population (151). The occurrence of the defined SNPs corresponds to the other studies, but in the control group the proportion of SNPs, mainly in the case of IL-1B+3953, is higher than in the published studies.

Only a few studies have been published until now about the connection between Interleukin-1 and MRONJ. *Bagan et al.* tested the amount of IL-1α, IL-1RA and IL-1β

cytokine in saliva among patients with MRONJ and without MRONJ (152). In their study, the values of IL-1β cytokine in saliva showed a significant difference in the MRONJ group compared to the group without MRONJ (152). *Barros Silva et al.* examined cell profile and proinflammatory markers in an animal model of BRONJ (153). IL-1β positive cells showed dose-dependent increases in the zoledronic acid-treated group in the study (153). *Endo et al.* published their animal study in 2017, which examined mice treated with intraperitoneal injected nitrogen-containing bisphosphonates (154). They found that LPS-induced histone decarboxylase induction is enhanced through increased production of IL-1α and IL-1β. In IL-1-KO mice, the inflammatory reaction induced by intraperitoneal nitrogen-containing bisphosphonates is very mild or undetectable (154, 155). This research group found in their investigation (published in 2009) that intraperitoneally, alone administered amino bisphosphonate caused no elevation in IL-1α, IL-1 β and TNFα serum levels (156). If the LPS injection was used 3 days after the amino bisphosphonate injection, increased serum levels of IL-1α and IL-1 β were detected (156). This study also notes that bone marrow and spleen cells taken from amino bisphosphonate-treated mice substantiate IL-1β spontaneously and its production is enhanced after a lipopolysaccharides injection (156). From the same research group, *Yamaguchi et al.* also described in their publication in 2000 that they measured HDC activity in mice injected with lipopolysaccharides from Escherichia coli and Prevotella intermedia (gram-negative bacteria have a role in periodontitis and endodontic infections) (155). If LPS and aminoBP were administered to mice, elevation in IL-1α and IL-1β serum levels and HDC activity in various tissues were detected (155). In IL-1 knock-out mice a small or undetectable immune response was experienced. Based on this investigation, the role of gram-negative bacteria arises in the pathomechanism of MRONJ (155). In a study, published in 2009, *Deng et al.* examined the proinflammatory cytokine production of Porphyromonas gingivalis and Tannerella forsythia (gram-negative bacteria have a role in periodontitis) infected macrophages in a mouse macrophage-like cell line in groups treated with nitrogen-containing bisphosphonate (alendronate) and non-nitrogen containing bisphosphonate (clodronate). In the group treated with nitrogen-containing bisphosphonate elevated IL-1β production was measured, in contrast, in the clodronate-treated group no IL-1β increase was detected (157).

IL-1α and IL-1β cytokines play a role in the initiation and regulation of the inflammatory process (62, 72). IL-1α and IL-1β incite the production of transcription factors, MAPKs, ERKs, interferon-regulating genes, cytokines, chemokines, interferons and prostaglandins, and the activation of T-helper cells and the migration of immune cells to tissues (72, 158). The IL-1A and IL-1B SNPs through structural changes in translated proteins cause changes in protein function and interactions with other proteins. The SNPs of the IL-1A and IL-1B genes can change IL-1α and IL-1β cytokine production and interactions with other proteins that can lead to an altered immune response to pro-inflammatory signals (84). IL-1, produced during the immune response, stimulates osteoclasts, and therefore affects bone metabolism (159, 160).

In the study, a significant correlation was found between two examined single polymorphisms and the presence of recurrences (62). In light of the results of this study, it can be assumed that the examined SNPs, through influencing the immune response and bone turnover, play a role in the occurrence of the relatively high relapse rate.

The limitations of this study are the relatively small number of patients, the attainability of quality patient data in databases, and the assumed multifactorial and unclear background of the disease (62). Within the possible limitations of this study, it can be assumed that the analysis of the defined SNPs of Interleukin-1 may help define the risk stratification of MRONJ after surgical therapy and provide available procedures to identify IL-1 polymorphisms in order to predict a higher risk of MRONJ relapses (62).

In the second study, the surgical therapy is supplemented with A-PRF addition. The hypothesis of this study is based on the fact that surgical therapy is the first choice among treatment options, however, operative therapy leads to healing in only 53.4-84.6% of the cases, and PRF is applied in several oral and maxillofacial procedures successfully to improve the recovery effectiveness (1, 54, 65). Another assumption of this study was that PRF application may also reduce the occurrence of recurrences. This supposition is based on the studies that described the development of recurrences after surgical therapy as associated with the type of healing among other factors (60, 66). Kang et al. in their study published in 2018 found that in the case of primary healing, the recurrence rate is 11.8% and in the case of secondary healing the recurrence rate is 45% (146). Recurrences and non-healing necrotic bone parts cause a significant decrease in patients' quality of life due to repeated intraoral or extraoral discharge, the loss of teeth, possible facial

deformities, and speaking and eating difficulties (1, 62). The treatment of relapses and non-healing necrotic bone parts after surgical therapy is a major challenge in clinical practice. Recurrences and non-healing necrotic bone parts that occur after surgical therapy require repeated or long-term antibiotic administration, which may cause antibiotic resistance increases and may necessitate the administration of antibiotic combinations. Recurrences diminish the amount of soft tissue that can be used for primary wound closure during recurrences. The scarring of the surgical site resulting from previous operations also makes the operation difficult. Because of this factor, the chance of wound dehiscence increases and the healing of the surgical site is reduced furthermore (62). Non-healing bone parts can lead to largely extended osteonecrosis, and may only be repaired with reconstructive operations, which pose a greater risk to patients.

The study involved 81 patients. The patients' data according to anamnestic and therapeutic data and necrosis features corresponded with the data of other studies that investigated the MRONJ (139, 141, 143-145, 161, 162).

The surgical site was healed in 83.6% of the cases and recurrence was detected in 40.57% of the patients who healed after the operation. The healing proportion and the recurrence rate correspond to the data in the literature (54, 55, 59). Stage improvement was observed in 100% of the group treated with PRF-supplemented surgical therapy, and 86.36% in the group treated with conventional surgical therapy. In the experimental group, the healing is 94.6%, and in the group treated with conventional surgical therapy, it is 75%. Recurrences occurred in 25.71% of the cases in the group treated with PRF-supplemented surgical therapy, and in 55.88% in the group treated with conventional surgical therapy. In the patient group, which was treated with PRF-supplemented surgical treatment, significantly better results were detected in terms of wound healing and stage improvement, and significantly lower recurrences were observed than in the control group (61).

Until now, few original articles and systematic reviews have been published on the application of PRF in the surgical therapy of MRONJ. The original articles are case reports and prospective cohort studies with the inclusion of 14-45 patients. All the studies described successful treatment with PRF supplementation and the cohort studies found an increase in treatment effectiveness regarding healing (163, 164). According to a systematic review published by *Bracher et al.* in 2021, the summarised results of studies

show that wound healing after PRF-supplemented surgical therapy 92.8% (162). *Muñoz-Salgado et al.* in their systematic review and meta-analysis described 94.3% complete healing after PRF-supplemented surgical treatment based on the summarized results of publications (161). The results of this study correspond to the outcomes of original articles and systematic reviews.

The exact mechanism of PRF's positive effect on the healing of MRONJ is unclear, only assumptions are available. *Ruggiero et al.* in their article hypothesised that this effect is due to PRF stimulating bone and mucosal healing (1). The effect of PRF is partly attributed to ensuring mechanical stability to wounds and partly to compensating the mechanisms which have a role in the development of the disease, for example, by stimulating bone modelling and local angiogenesis, and by regulating local immune mechanisms. According to theories, bone remodeling inhibition, through the direct effect of bisphosphonates on osteoclast and through the effect of denosumab on RANK-RANKL binding, plays a role in developing MRONJ (11). PRF contains growth factors in the alpha granules' platelets, which regulate the signalling pathways of mature mesenchymal stem cells, osteoblasts and endothelial cells through stimulating osteoid modelling and intercellular matrix production (165, 166). Nitrogen-containing BPs decrease the production of VEGF and impede the proliferation, migration and adhesion of endothelial cells (34, 167). PRF contains, among other growth factors, such as VEGF, FGFb and PDGF. Infections, inflammation and immune dysfunction also have an assumed role in the development of MRONJ. The fibrin and the fibrin degradation products (FDP) in PRF stimulate transendothelial cell migration, neutrophil granulocytes phagocytosis and intracellular enzymatic breakdown mechanisms in immune cells, and through them incite local immune mechanisms (34, 167). Nitrogen-containing BPs have a direct toxic effect on epithelial cells and macrophages, thus damaging the integrity of oral mucous membranes and decreasing healing (136). PRF membranes create a matrix and provide mechanical stability to wounds, and thus presumably improve the exfoliating ability of wounds despite impaired mucosal healing (168).

The limitations of this study are the low number of cases and the multifactorial and unclear risk and prognostic factors of the disease. Going further, we plan to continue the examination with 100 patients included in each group.

6. Conclusions

The medication-related osteonecrosis of the jaw can significantly reduce patients' quality of life because of the pain, the constant discharge from extraoral and intraoral fistulas, paresthesia, halitosis, difficulty in chewing and speaking, oro-antral and oro-nasal communications and facial deformity (1, 15). MRONJ can cause abscesses or phlegmon, which are life-threatening complications of the disease.

From the first description of the disease in 2004, many studies have been published about the pathomechanism, the risk and prognostic factors and the treatment options and strategies of the disease. Despite these investigations, the exact pathomechanism process of the disease, and the risk and prognostic factors have not been clarified. The treatment that provides guaranteed success and recovery in 100% of the cases is also unknown. Therefore, the process of the pathomechanism, the risk and prognostic factors, and the treatment options require further investigation. The clarification of the pathomechanism, and the risk and prognostic factors may help in the risk classification of patients who receive antiresorptive therapy and may contribute to preventing MRONJ and developing new treatment options. The new, supplement procedures that are applied in addition to surgical therapy, can improve the effectiveness of surgical treatment regarding healing and recurrences.

In this thesis, two clinical studies are presented that I conducted during my doctoral studies. The "Interleukin 1" study hypotheses that the IL-1A−889 (rs18000587) and IL-1B+3953 (rs1143634) SNPs' have a role in the development and prognosis of MRONJ. The results of the study's four aims examination are:

*Aim/Result I/1:*no association was found between the carrying of either examined SNP and the development of MRONJ.

*Aim/Result I/2:*no connection was discerned between the presence of SNPs and the stage improvement and healing, but a correlation was detected between the occurrence of the SNPs and the recurrences of MRONJ in several examined genetic aspects:

- **A.** A significant association was detected between the carrying of at least one of the examined SNPs and recurrences.
- **B.** A significant connection was also shown between separate carrying of the IL-1A-889 T SNP/ IL-1B+3953T SNP/both IL-1A-889 T and IL-1B+3953T SNPs and the recurrences and the number of recurrences.

- **C.** A significant correlation was observed between the carrying of the IL-1B+3953 T SNP on all or one or none copies of the IL-1B gene and recurrences.

In two genetic aspects no correlation was detected:

- **(D.** no association was found between the presence of the IL-1A-889 T SNP on all or one or none copies of the IL-1A gene and recurrence)
- **(E.** No correlation was observed between the 9 examined combinations and recurrences either)

Aim/Result I/3: no association was detected between the SNPs and the stage at diagnosis, the localisation of the affected area and tooth extraction before the development of MRONJ.

Aim/Result I/4: no anamnestic or therapeutic data or necrosis feature has shown an effect in combination with SNPs on the prognosis of MRONJ.

The "PRF" study hypothesises that the application of A-PRF improves the effectiveness of surgical therapy in terms of wound healing and the prognosis of the disease. The results of the second study's two aims examination are:

AimResult II/1: PRF-supplemented surgical therapy compared to conventional operations significantly enhanced the stage improvement and healing after the intervention and significantly decreased relapse rates.

(Aim/Results II/2). No necrosis features or anamnestic or therapeutic data showed an effect on the outcome in the case of PRF-supplemented surgical treatment.

Until now, no studies have been published about the Interleukin 1A and 1B SNPs' role in the development and prognosis of MRONJ (62). Until now, few articles have been published on the use of PRF in the surgical therapy of MRONJ. Most of these studies were case reports and case-control studies and included low number of patients The results of these articles correspond to the observations of this study. Further investigations are required to clarify the defined SNPs' role in the development and prognosis of MRONJ and the effect of PRF application on the surgical site's healing and recurrences of MRONJ after surgical treatment.

7. Summary

The MRONJ can cause the patient's significant reduction in quality of life, especially in case of recurrences, can lead to facial deformity and life-threatening complications (1). Until now, the exact process of pathomechanism, the risk and prognostic factors of the disease, the treatment that provides recovery in 100% are not clarified. The process of pathomechanism, the risk and prognostic factors and treatment options request further investigations.

The „Interleukin 1" study aims to investigate the role of the IL-1A−889 (rs 18000587) and IL-1B+3953 (rs 1143634) SNPs in the development and prognosis of MRONJ. No correlation was found between the occurrence of either examined SNP and the development of MRONJ. Also, no connection was observed between the presence of SNPs and the stage improvement and healing after surgical therapy. On the contrary, an association was detected between the SNP's occurrence and the MRONJ's recurrences in several examined aspects. No connection was discerned between the SNPs and the necrosis features. No anamnestic or therapeutic data or necrosis feature has shown an effect in combination with SNPs on the prognosis of the MRONJ.

The „PRF" study aimed to observe the effect of A-PRF-supplemented surgical therapy on the prognosis of MRONJ. The PRF-supplemented surgical therapy compared to the conventional operation significantly increased the stage improvement and healing after the intervention and significantly decreased relapse rates (61). No necrosis features or anamnestic or therapeutic data showed an effect on the outcome in the case of PRF-supplemented surgical treatment.

Interleukin 1 SNPs have never been tested in the development and prognosis of MRONJ based on the literature. Only a few animal and human studies have been conducted until now about the relationship between Interleukin 1 and MRONJ. A few articles have been published about the application of PRF in the MRONJ's surgical therapy. The results of these articles correspond to the observations of this study.

The results of these two studies in the future may help in the risk classification of patients and preventing MRONJ, assist in the early diagnosis making and prevent the development of high-stage MRONJ and succor to develop new treatment options and strategies which are more effective than the now-known procedures.

8. New statements of the thesis

Based on the publications in the literature, Interleukin 1 SNPs have never been tested in the development and prognosis of MRONJ. The results of the “Interleukin 1” study, described in this thesis are:

- A correlation was detected between the occurrence of the SNPs and the recurrences of MRONJ (62)
    - A significant association was detected between the carrying of at least one of the examined SNPs and recurrences.
    - A significant connection was also shown between separate carrying of the IL-1A-889 T SNP/ IL-1B+3953T SNP/both IL-1A-889 T and IL-1B+3953T SNPs and the recurrences and the number of recurrences.
    - A significant correlation was observed between the carrying of the IL-1B+3953 T SNP on all or one or none copies of the IL-1B gene and
- No association was found between the carrying of either examined SNP and the development of MRONJ
- No association was detected between the SNPs and the stage at diagnosis, the localisation of the affected area and tooth extraction before the development of MRONJ
- No anamnestic or therapeutic data or necrosis feature has shown an effect in combination with SNPs on the prognosis of MRONJ

In the “PRF” study which is randomized controlled trial with a higher number of patients than in publications, we found:

- A-PRF-supplemented surgical therapy compared to conventional operations significantly enhanced the stage improvement and healing after the intervention and significantly decreased relapse rates
- No necrosis features, anamnestic or therapeutic data showed an effect on the outcome in the case of PRF-supplemented surgical treatment

9. References

1. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. J Oral Maxillofac Surg. 2022;80(5):920-43.
2. Koth VS, Figueiredo MA, Salum FG, Cherubini K. Bisphosphonate-related osteonecrosis of the jaw: from the sine qua non condition of bone exposure to a non-exposed BRONJ entity. Dentomaxillofac Radiol. 2016;45(7):20160049.
3. Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg. 2003;61(9):1115-7.
4. American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws. J Oral Maxillofac Surg. 2007;65(3):369-76.
5. Khosla S, Burr D, Cauley J, Dempster DW, Ebeling PR, Felsenberg D, et al. Bisphosphonate-associated osteonecrosis of the jaw: report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res. 2007;22(10):1479-91.
6. Marx RE. Bisphosphonate-induced osteonecrosis of the jaws: a challenge, a responsibility, and an opportunity. Int J Periodontics Restorative Dent. 2008;28(1):5-6.
7. Kelleher FC, McKenna M, Collins C, Brady G, Collins I, Crown J. Bisphosphonate induced osteonecrosis of the jaws: unravelling uncertainty in disease causality. Acta Oncol. 2007;46(5):702-4.
8. Ruggiero SL, Dodson TB, Assael LA, Landesberg R, Marx RE, Mehrotra B. American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws--2009 update. J Oral Maxillofac Surg. 2009;67(5 Suppl):2-12.
9. Kendler DL, Cosman F, Stad RK, Ferrari S. Denosumab in the Treatment of Osteoporosis: 10 Years Later: A Narrative Review. Adv Ther. 2022;39(1):58-74.
10. Noble JA, McKenna MJ, Crowley RK. Should denosumab treatment for osteoporosis be continued indefinitely? Ther Adv Endocrinol Metab. 2021;12:20420188211010052.

11. Aghaloo TL, Felsenfeld AL, Tetradis S. Osteonecrosis of the jaw in a patient on Denosumab. J Oral Maxillofac Surg. 2010;68(5):959-63.
12. Tagliamento M, Boutros C, Viansone A, Brau JJ, Descols P. Denosumab related osteonecrosis of the jaw: Unusual pattern with periosteal reaction. Eur J Cancer. 2022;166:33-7.
13. Hellstein JW, Adler RA, Edwards B, Jacobsen PL, Kalmar JR, Koka S, et al. Managing the care of patients receiving antiresorptive therapy for prevention and treatment of osteoporosis: executive summary of recommendations from the American Dental Association Council on Scientific Affairs. J Am Dent Assoc. 2011;142(11):1243-51.
14. Estilo CL, Fornier M, Farooki A, Carlson D, Bohle G, 3rd, Huryn JM. Osteonecrosis of the jaw related to bevacizumab. J Clin Oncol. 2008;26(24):4037-8.
15. Sacco R, Woolley J, Patel G, Calasans-Maia MD, Yates J. Systematic review of medication related osteonecrosis of the jaw (MRONJ) in patients undergoing only antiangiogenic drug therapy: surgery or conservative therapy? Br J Oral Maxillofac Surg. 2022;60(2):e216-e30.
16. Hamadeh IS, Ngwa BA, Gong Y. Drug induced osteonecrosis of the jaw. Cancer Treat Rev. 2015;41(5):455-64.
17. Ruggiero SL, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, et al. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw--2014 update. J Oral Maxillofac Surg. 2014;72(10):1938-56.
18. Fleisch H, Russell RG, Bisaz S, Mühlbauer R. [Influence of diphosphonates on the deposition and dissolution ofccalcium phosphate in vitro and in vivo]. Helv Physiol Pharmacol Acta. 1968;26(3):Cr345-6.
19. Roelofs AJ, Ebetino FH, Reszka AA, Russell RGG, Rogers MJ. Chapter 81 - Bisphosphonates: Mechanisms of Action. In: Bilezikian JP, Raisz LG, Martin TJ, editors. Principles of Bone Biology (Third Edition). San Diego: Academic Press; 2008. p. 1737-67.
20. Bojtor B, Vaszilko M, Armos R, Tobias B, Podani J, Szentpeteri S, et al. Analysis of SIRT1 Gene SNPs and Clinical Characteristics in Medication-Related Osteonecrosis of the Jaw. Int J Mol Sci. 2024;25(7).

21. Narayanan P. Denosumab: A comprehensive review. South Asian J Cancer. 2013;2(4):272-7.
22. Lim SY, Bolster MB. Profile of romosozumab and its potential in the management of osteoporosis. Drug Des Devel Ther. 2017;11:1221-31.
23. Tetradis S, Allen MR, Ruggiero SL. Pathophysiology of Medication-Related Osteonecrosis of the Jaw-A Minireview. JBMR Plus. 2023;7(8):e10785.
24. Wehrhan F, Weber M, Neukam FW, Geppert CI, Kesting M, Preidl RHM. Fluorescence-guided bone resection: A histological analysis in medication-related osteonecrosis of the jaw. J Craniomaxillofac Surg. 2019;47(10):1600-7.
25. Wehrhan F, Hyckel P, Guentsch A, Nkenke E, Stockmann P, Schlegel KA, et al. Bisphosphonate-associated osteonecrosis of the jaw is linked to suppressed TGFβ1-signaling and increased Galectin-3 expression: a histological study on biopsies. J Transl Med. 2011;9:102.
26. He L, Sun X, Liu Z, Qiu Y, Niu Y. Pathogenesis and multidisciplinary management of medication-related osteonecrosis of the jaw. Int J Oral Sci. 2020;12(1):30.
27. Manzano-Moreno FJ, Ramos-Torrecillas J, Melguizo-Rodríguez L, Illescas-Montes R, Ruiz C, García-Martínez O. Bisphosphonate Modulation of the Gene Expression of Different Markers Involved in Osteoblast Physiology: Possible Implications in Bisphosphonate-Related Osteonecrosis of the Jaw. Int J Med Sci. 2018;15(4):359-67.
28. Spector JA, Mathy JA, Warren SM, Nacamuli RP, Song HM, Lenton K, et al. FGF-2 acts through an ERK1/2 intracellular pathway to affect osteoblast differentiation. Plast Reconstr Surg. 2005;115(3):838-52.
29. Bassan Marinho Maciel G, Marinho Maciel R, Linhares Ferrazzo K, Cademartori Danesi C. Etiopathogenesis of medication-related osteonecrosis of the jaws: a review. J Mol Med (Berl). 2024;102(3):353-64.
30. Hallmer F, Bjørnland T, Andersson G, Becktor JP, Kristoffersen AK, Enersen M. Bacterial diversity in medication-related osteonecrosis of the jaw. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017;123(4):436-44.
31. Mawardi H, Giro G, Kajiya M, Ohta K, Almazrooa S, Alshwaimi E, et al. A role of oral bacteria in bisphosphonate-induced osteonecrosis of the jaw. J Dent Res. 2011;90(11):1339-45.

32. Soundia A, Hadaya D, Esfandi N, de Molon RS, Bezouglaia O, Dry SM, et al. Osteonecrosis of the jaws (ONJ) in mice after extraction of teeth with periradicular disease. Bone. 2016;90:133-41.

33. Santini D, Vespasiani Gentilucci U, Vincenzi B, Picardi A, Vasaturo F, La Cesa A, et al. The antineoplastic role of bisphosphonates: from basic research to clinical evidence. Ann Oncol. 2003;14(10):1468-76.

34. Wood J, Bonjean K, Ruetz S, Bellahcène A, Devy L, Foidart JM, et al. Novel antiangiogenic effects of the bisphosphonate compound zoledronic acid. J Pharmacol Exp Ther. 2002;302(3):1055-61.

35. Gkouveris I, Hadaya D, Soundia A, Bezouglaia O, Chau Y, Dry SM, et al. Vasculature submucosal changes at early stages of osteonecrosis of the jaw (ONJ). Bone. 2019;123:234-45.

36. Bastos P, Patel V, Festy F, Hosny N, Cook RJ. In-vivo imaging of the microvasculature of the soft tissue margins of osteonecrotic jaw lesions. Br Dent J. 2017;223(9):699-705.

37. Filleul O, Crompot E, Saussez S. Bisphosphonate-induced osteonecrosis of the jaw: a review of 2,400 patient cases. J Cancer Res Clin Oncol. 2010;136(8):1117-24.

38. Zhang Q, Yu W, Lee S, Xu Q, Naji A, Le AD. Bisphosphonate Induces Osteonecrosis of the Jaw in Diabetic Mice via NLRP3/Caspase-1-Dependent IL-1β Mechanism. J Bone Miner Res. 2015;30(12):2300-12.

39. Rao NJ, Yu RQ, Wang JY, Helm A, Zheng LW. Effect of Periapical Diseases in Development of MRONJ in Immunocompromised Mouse Model. Biomed Res Int. 2019;2019:1271492.

40. Simon MJ, Niehoff P, Kimmig B, Wiltfang J, Açil Y. Expression profile and synthesis of different collagen types I, II, III, and V of human gingival fibroblasts, osteoblasts, and SaOS-2 cells after bisphosphonate treatment. Clin Oral Investig. 2010;14(1):51-8.

41. Sandro Pereira da Silva J, Pullano E, Raje NS, Troulis MJ, August M. Genetic predisposition for medication-related osteonecrosis of the jaws: a systematic review. Int J Oral Maxillofac Surg. 2019;48(10):1289-99.

42. Yang G, Singh S, Chen Y, Hamadeh IS, Langaee T, McDonough CW, et al. Pharmacogenomics of osteonecrosis of the jaw. Bone. 2019;124:75-82.

43. Laputková G, Talian I, Schwartzová V. Medication-Related Osteonecrosis of the Jaw: A Systematic Review and a Bioinformatic Analysis. Int J Mol Sci. 2023;24(23).
44. Ohga N, Sato J, Asaka T, Morimoto M, Yamazaki Y, Kitagawa Y. Successful conservative treatment of jaw osteonecrosis caused by denosumab in patients with multiple bone metastasis. J Oral Sci. 2018;60(1):159-62.
45. Di Fede O, Del Gaizo C, Panzarella V, La Mantia G, Tozzo P, Di Grigoli A, et al. Ozone Infiltration for Osteonecrosis of the Jaw Therapy: A Case Series. J Clin Med. 2022;11(18).
46. Inchingolo F, Cantore S, Dipalma G, Georgakopoulos I, Almasri M, Gheno E, et al. Platelet rich fibrin in the management of medication-related osteonecrosis of the jaw: a clinical and histopathological evaluation. J Biol Regul Homeost Agents. 2017;31(3):811-6.
47. Inchingolo AM, Patano A, Di Pede C, Inchingolo AD, Palmieri G, de Ruvo E, et al. Autologous Tooth Graft: Innovative Biomaterial for Bone Regeneration. Tooth Transformer(®) and the Role of Microbiota in Regenerative Dentistry. A Systematic Review. J Funct Biomater. 2023;14(3).
48. Khan AA, Morrison A, Hanley DA, Felsenberg D, McCauley LK, O'Ryan F, et al. Diagnosis and management of osteonecrosis of the jaw: a systematic review and international consensus. J Bone Miner Res. 2015;30(1):3-23.
49. Graziani F, Vescovi P, Campisi G, Favia G, Gabriele M, Gaeta GM, et al. Resective surgical approach shows a high performance in the management of advanced cases of bisphosphonate-related osteonecrosis of the jaws: a retrospective survey of 347 cases. J Oral Maxillofac Surg. 2012;70(11):2501-7.
50. El-Rabbany M, Blanas N, Sutherland S, Lam DK, Shah PS, Azarpazhooh A. Surgical Therapy in Patients With Medication-Related Osteonecrosis of the Jaw Is Associated With Disease Resolution and Improved Quality of Life: A Prospective Cohort Study. J Oral Maxillofac Surg. 2022;80(6):1084-93.
51. El-Rabbany M, Lam DK, Shah PS, Azarpazhooh A. Surgical Management of Medication-Related Osteonecrosis of the Jaw Is Associated With Improved Disease Resolution: A Retrospective Cohort Study. J Oral Maxillofac Surg. 2019;77(9):1816-22.

52. Moll S, Mueller S, Meier JK, Reichert TE, Ettl T, Klingelhöffer C. Patients' quality of life improves after surgical intervention of stage III medication-related osteonecrosis of the jaw. Oral Maxillofac Surg. 2021;25(3):359-66.
53. Otto S, Pautke C, Van den Wyngaert T, Niepel D, Schiødt M. Medication-related osteonecrosis of the jaw: Prevention, diagnosis and management in patients with cancer and bone metastases. Cancer Treat Rev. 2018;69:177-87.
54. Coropciuc R, Coopman R, Garip M, Gielen E, Politis C, Van den Wyngaert T, et al. Risk of medication-related osteonecrosis of the jaw after dental extractions in patients receiving antiresorptive agents - A retrospective study of 240 patients. Bone. 2023;170:116722.
55. Kang MH, Lee DK, Kim CW, Song IS, Jun SH. Clinical characteristics and recurrence-related factors of medication-related osteonecrosis of the jaw. J Korean Assoc Oral Maxillofac Surg. 2018;44(5):225-31.
56. Wick A, Bankosegger P, Otto S, Hohlweg-Majert B, Steiner T, Probst F, et al. Risk factors associated with onset of medication-related osteonecrosis of the jaw in patients treated with denosumab. Clin Oral Investig. 2022;26(3):2839-52.
57. AlRowis R, Aldawood A, AlOtaibi M, Alnasser E, AlSaif I, Aljaber A, et al. Medication-Related Osteonecrosis of the Jaw (MRONJ): A Review of Pathophysiology, Risk Factors, Preventive Measures and Treatment Strategies. Saudi Dent J. 2022;34(3):202-10.
58. Ottesen C, Schiodt M, Gotfredsen K. Efficacy of a high-dose antiresorptive drug holiday to reduce the risk of medication-related osteonecrosis of the jaw (MRONJ): A systematic review. Heliyon. 2020;6(4):e03795.
59. Chen S, Ren H, He Y, An J, Zhang Y. Recurrence-Related Factors of Medication-Related Osteonecrosis of the Jaw: A Five-Year Experience. J Oral Maxillofac Surg. 2021;79(12):2472-81.
60. Szentpéteri S, Restár L, Németh Z, Vaszilkó M. [Prognostic factors of the medication-related osteonecrosis of the jaw]. Orv Hetil. 2020;161(8):283-9.
61. Szentpeteri S, Schmidt L, Restar L, Csaki G, Szabo G, Vaszilko M. The Effect of Platelet-Rich Fibrin Membrane in Surgical Therapy of Medication-Related Osteonecrosis of the Jaw. J Oral Maxillofac Surg. 2020;78(5):738-48.

62. Szentpeteri S, Kosa J, Juhasz HD, Deak G, Nemeth Z, Lakatos P, et al. Examination of certain single-nucleotide polymorphisms of interleukins 1A and 1B in medication-related osteonecrosis of the jaw — An ambirectional cohort study. Journal of Cranio-Maxillofacial Surgery. 2024.

63. Voss PJ, Joshi Oshero J, Kovalova-Müller A, Veigel Merino EA, Sauerbier S, Al-Jamali J, et al. Surgical treatment of bisphosphonate-associated osteonecrosis of the jaw: technical report and follow up of 21 patients. J Craniomaxillofac Surg. 2012;40(8):719-25.

64. Stockmann P, Vairaktaris E, Wehrhan F, Seiss M, Schwarz S, Spriewald B, et al. Osteotomy and primary wound closure in bisphosphonate-associated osteonecrosis of the jaw: a prospective clinical study with 12 months follow-up. Support Care Cancer. 2010;18(4):449-60.

65. On S-W, Seoung-Won Cho, Soo-Hwan Byun, and Byoung-Eun Yang. Various Therapeutic Methods for the Treatment of Medication-Related Osteonecrosis of the Jaw (MRONJ) and Their Limitations: A Narrative Review on New Molecular and Cellular Therapeutic Approaches Antioxidants (Basel). 2021;10(5):680.

66. Wutzl A, Pohl S, Sulzbacher IS, R., Lauer G, Ewers R, Drach J, et al. Factors influencing surgical treatment of bisphosphonate-related osteonecrosis of the jaws. Head Neck. 2012;34(2):194-200.

67. Vaszilko M, Kovacs E, Restar L, Balla B, Cseplo K, Kosa J, et al. Potential significance of antiestrogen therapy in the development of bisphosphonate related osteonecrosis of the jaw. J Craniomaxillofac Surg. 2014;42(8):1932-6.

68. Yamada SI, Kurita H, Kondo E, Suzuki S, Nishimaki F, Yoshimura N, et al. Treatment outcomes and prognostic factors of medication-related osteonecrosis of the jaw: a case- and literature-based review. Clin Oral Investig. 2019;23(8):3203-11.

69. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev. 2018;281(1):8-27.

70. di Giovine FS, Duff GW. Interleukin 1: the first interleukin. Immunol Today. 1990;11(1):13-20.

71. Malik A, Kanneganti TD. Function and regulation of IL-1α in inflammatory diseases and cancer. Immunol Rev. 2018;281(1):124-37.

72. Cavalli G, Colafrancesco S, Emmi G, Imazio M, Lopalco G, Maggio MC, et al. Interleukin 1α: a comprehensive review on the role of IL-1α in the pathogenesis and treatment of autoimmune and inflammatory diseases. Autoimmun Rev. 2021;20(3):102763.
73. Abbate A, Toldo S, Marchetti C, Kron J, Van Tassell BW, Dinarello CA. Interleukin-1 and the Inflammasome as Therapeutic Targets in Cardiovascular Disease. Circ Res. 2020;126(9):1260-80.
74. Pfeiler S, Winkels H, Kelm M, Gerdes N. IL-1 family cytokines in cardiovascular disease. Cytokine. 2019;122:154215.
75. Arvizu-Rivera RI, Azpiri-Lopez JR, Colunga-Pedraza IJ, Cardenas-de la Garza JA, Vera-Pineda R, Garcia-Arellano G, et al. Carotid Plaque Is Not Associated With Increased Levels of Interleukin 1, Interleukin 6, and Tumor Necrosis Factor α in Rheumatoid Arthritis. J Clin Rheumatol. 2021;27(8):e602-e5.
76. Dinarello CA. The IL-1 family of cytokines and receptors in rheumatic diseases. Nat Rev Rheumatol. 2019;15(10):612-32.
77. Díaz-González F, Hernández-Hernández MV. Rheumatoid arthritis. Med Clin (Barc). 2023;161(12):533-42.
78. Cheng R, Wu Z, Li M, Shao M, Hu T. Interleukin-1β is a potential therapeutic target for periodontitis: a narrative review. Int J Oral Sci. 2020;12(1):2.
79. Yang NY, Zhou Y, Zhao HY, Liu XY, Sun Z, Shang JJ. Increased interleukin 1α and interleukin 1β expression is involved in the progression of periapical lesions in primary teeth. BMC Oral Health. 2018;18(1):124.
80. Matsuo T, Ebisu S, Nakanishi T, Yonemura K, Harada Y, Okada H. Interleukin-1 alpha and interleukin-1 beta periapical exudates of infected root canals: correlations with the clinical findings of the involved teeth. J Endod. 1994;20(9):432-5.
81. Sorenson A, Hresko K, Butcher S, Pierce S, Tramontina V, Leonardi R, et al. Expression of Interleukin-1 and temporomandibular disorder: Contemporary review of the literature. Cranio. 2018;36(4):268-72.
82. Brailo V, Vucicevic-Boras V, Lukac J, Biocina-Lukenda D, Zilic-Alajbeg I, Milenovic A, et al. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. Med Oral Patol Oral Cir Bucal. 2012;17(1):e10-5.

83. Asensi V, Alvarez V, Valle E, Meana A, Fierer J, Coto E, et al. IL-1 alpha (-889) promoter polymorphism is a risk factor for osteomyelitis. Am J Med Genet A. 2003;119a(2):132-6.
84. Khazim K, Azulay EE, Kristal B, Cohen I. Interleukin 1 gene polymorphism and susceptibility to disease. Immunol Rev. 2018;281(1):40-56.
85. Behzadi P, Sameer AS, Nissar S, Banday MZ, Gajdács M, García-Perdomo HA, et al. The Interleukin-1 (IL-1) Superfamily Cytokines and Their Single Nucleotide Polymorphisms (SNPs). J Immunol Res. 2022;2022:2054431.
86. Zhang AQ, Pan W, Gao JW, Yue CL, Zeng L, Gu W, et al. Associations between interleukin-1 gene polymorphisms and sepsis risk: a meta-analysis. BMC Med Genet. 2014;15:8.
87. Tsezou A, Poultsides L, Kostopoulou F, Zintzaras E, Satra M, Kitsiou-Tzeli S, et al. Influence of interleukin 1alpha (IL-1alpha), IL-4, and IL-6 polymorphisms on genetic susceptibility to chronic osteomyelitis. Clin Vaccine Immunol. 2008;15(12):1888-90.
88. Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent. 2001;10(4):225-8.
89. Alves R, Grimalt R. A Review of Platelet-Rich Plasma: History, Biology, Mechanism of Action, and Classification. Skin Appendage Disord. 2018;4(1):18-24.
90. Ghanaati S, Herrera-Vizcaino C, Al-Maawi S, Lorenz J, Miron RJ, Nelson K, et al. Fifteen Years of Platelet Rich Fibrin in Dentistry and Oromaxillofacial Surgery: How High is the Level of Scientific Evidence? J Oral Implantol. 2018;44(6):471-92.
91. Gibble JW, Ness PM. Fibrin glue: the perfect operative sealant? Transfusion. 1990;30(8):741-7.
92. Rosenthal AR, Egbert PR, Harbury C, Hopkins JL, Rubenstein E. Use of platelet-fibrinogen-thrombin mixture to seal experimental penetrating corneal wounds. Albrecht Von Graefes Arch Klin Exp Ophthalmol. 1978;207(2):111-5.
93. Knighton DR, Ciresi KF, Fiegel VD, Austin LL, Butler EL. Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). Ann Surg. 1986;204(3):322-30.
94. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85(6):638-46.

95. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):e37-44.
96. Saluja H, Dehane V, Mahindra U. Platelet-Rich fibrin: A second generation platelet concentrate and a new friend of oral and maxillofacial surgeons. Ann Maxillofac Surg. 2011;1(1):53-7.
97. Kumar KR, Genmorgan K, Abdul Rahman SM, Rajan MA, Kumar TA, Prasad VS. Role of plasma-rich fibrin in oral surgery. J Pharm Bioallied Sci. 2016;8(Suppl 1):S36-s8.
98. Miron RJ, Zucchelli G, Pikos MA, Salama M, Lee S, Guillemette V, et al. Use of platelet-rich fibrin in regenerative dentistry: a systematic review. Clin Oral Investig. 2017;21(6):1913-27.
99. 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(7):1266-77.
100. Shashank B, Bhushan M. Injectable Platelet-Rich Fibrin (PRF): The newest biomaterial and its use in various dermatological conditions in our practice: A case series. J Cosmet Dermatol. 2021;20(5):1421-6.
101. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost. 2004;91(1):4-15.
102. Everts PA, Knape JT, Weibrich G, Schönberger JP, Hoffmann J, Overdevest EP, et al. Platelet-rich plasma and platelet gel: a review. J Extra Corpor Technol. 2006;38(2):174-87.
103. Obana KK, Schallmo MS, Hong IS, Ahmad CS, Moorman CT, 3rd, Trofa DP, et al. Current Trends in Orthobiologics: An 11-Year Review of the Orthopaedic Literature. Am J Sports Med. 2022;50(11):3121-9.
104. Tunalı M, Özdemir H, Küçükodacı Z, Akman S, Yaprak E, Toker H, et al. A novel platelet concentrate: titanium-prepared platelet-rich fibrin. Biomed Res Int. 2014;2014:209548.

105. Caruana A, Savina D, Macedo JP, Soares SC. From Platelet-Rich Plasma to Advanced Platelet-Rich Fibrin: Biological Achievements and Clinical Advances in Modern Surgery. Eur J Dent. 2019;13(2):280-6.
106. Pavlovic V, Ciric M, Jovanovic V, Trandafilovic M, Stojanovic P. Platelet-rich fibrin: Basics of biological actions and protocol modifications. Open Med (Wars). 2021;16(1):446-54.
107. Shah R, M GT, Thomas R, Mehta DS. An Update on the Protocols and Biologic Actions of Platelet Rich Fibrin in Dentistry. Eur J Prosthodont Restor Dent. 2017;25(2):64-72.
108. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol. 2009;27(3):158-67.
109. Miron RJ, Fujioka-Kobayashi M, Hernandez M, Kandalam U, Zhang Y, Ghanaati S, et al. Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry? Clin Oral Investig. 2017;21(8):2619-27.
110. Miron RJ, Fujioka-Kobayashi M, Sculean A, Zhang Y. Optimization of platelet-rich fibrin. Periodontol 2000. 2024;94(1):79-91.
111. Zheng X, Yan X, Cheng K, Feng M, Wang Y, Xiao B. Exploration of proper heating protocol for injectable horizontal platelet-rich fibrin gel. Int J Implant Dent. 2022;8(1):36.
112. Rosella D, Papi P, Giardino R, Cicalini E, Piccoli L, Pompa G. Medication-related osteonecrosis of the jaw: Clinical and practical guidelines. J Int Soc Prev Community Dent. 2016;6(2):97-104.
113. Campisi G, Fedele S, Fusco V, Pizzo G, Di Fede O, Bedogni A. Epidemiology, clinical manifestations, risk reduction and treatment strategies of jaw osteonecrosis in cancer patients exposed to antiresorptive agents. Future Oncol. 2014;10(2):257-75.
114. Martins AS, Correia JA, Salvado F, Caldas C, Santos N, Capelo A, et al. Relevant factors for treatment outcome and time to healing in medication-related osteonecrosis of the jaws - A retrospective cohort study. J Craniomaxillofac Surg. 2017;45(10):1736-42.
115. Brodzikowska A, Górska R, Kowalski J. Interleukin-1 Genotype in Periodontitis. Arch Immunol Ther Exp (Warsz). 2019;67(6):367-73.

116. Okuyama K, Hayashida S, Rokutanda S, Kawakita A, Soutome S, Sawada S, et al. Surgical strategy for medication-related osteonecrosis of the jaw (MRONJ) on maxilla: A multicenter retrospective study. J Dent Sci. 2021;16(3):885-90.
117. On SW, Cho SW, Byun SH, Yang BE. Various Therapeutic Methods for the Treatment of Medication-Related Osteonecrosis of the Jaw (MRONJ) and Their Limitations: A Narrative Review on New Molecular and Cellular Therapeutic Approaches. Antioxidants (Basel). 2021;10(5).
118. Fan Y, Perez K, Dym H. Clinical Uses of Platelet-Rich Fibrin in Oral and Maxillofacial Surgery. Dent Clin North Am. 2020;64(2):291-303.
119. Schwech N, Nilsson J, Gabre P. Incidence and risk factors for medication-related osteonecrosis after tooth extraction in cancer patients-A systematic review. Clin Exp Dent Res. 2023;9(1):55-65.
120. Dodson TB. The Frequency of Medication-related Osteonecrosis of the Jaw and its Associated Risk Factors. Oral Maxillofac Surg Clin North Am. 2015;27(4):509-16.
121. McGowan K, McGowan T, Ivanovski S. Risk factors for medication-related osteonecrosis of the jaws: A systematic review. Oral Dis. 2018;24(4):527-36.
122. Moreno Rabie C, García-Larraín S, Contreras Diez de Medina D, Cabello-Salazar I, Fontenele RC, Van den Wyngaert T, et al. How does the clinical and tomographic appearance of MRONJ influences its treatment prognosis? Dentomaxillofac Radiol. 2023;52(8):20230304.
123. Wei LY, Kok SH, Lee YC, Chiu WY, Wang JJ, Cheng SJ, et al. Prognosis of medication-related osteonecrosis of the jaws in metastatic prostate cancer patients. Oral Dis. 2022;28(1):182-92.
124. Yang G, Collins JM, Rafiee R, Singh S, Langaee T, McDonough CW, et al. SIRT1 Gene SNP rs932658 Is Associated With Medication-Related Osteonecrosis of the Jaw. J Bone Miner Res. 2021;36(2):347-56.
125. Sarasquete ME, García-Sanz R, Marín L, Alcoceba M, Chillón MC, Balanzategui A, et al. Bisphosphonate-related osteonecrosis of the jaw is associated with polymorphisms of the cytochrome P450 CYP2C8 in multiple myeloma: a genome-wide single nucleotide polymorphism analysis. Blood. 2008;112(7):2709-12.

126. Nicoletti P, Cartsos VM, Palaska PK, Shen Y, Floratos A, Zavras AI. Genomewide pharmacogenetics of bisphosphonate-induced osteonecrosis of the jaw: the role of RBMS3. Oncologist. 2012;17(2):279-87.

127. Katz J, Gong Y, Salmasinia D, Hou W, Burkley B, Ferreira P, et al. Genetic polymorphisms and other risk factors associated with bisphosphonate induced osteonecrosis of the jaw. Int J Oral Max Surg. 2011;40(6):605-11.

128. Stockmann P, Nkenke E, Englbrecht M, Schlittenbauer T, Wehrhan F, Rauh C, et al. Major histocompatibility complex class II polymorphisms are associated with the development of anti-resorptive agent-induced osteonecrosis of the jaw. Journal of Cranio-Maxillofacial Surgery. 2013;41(1):71-5.

129. Guo Z, Cui W, Que L, Li C, Tang X, Liu J. Pharmacogenetics of medication-related osteonecrosis of the jaw: a systematic review and meta-analysis. Int J Oral Maxillofac Surg. 2020;49(3):298-309.

130. English BC, Baum CE, Adelberg DE, Sissung TM, Kluetz PG, Dahut WL, et al. A SNP in CYP2C8 is not associated with the development of bisphosphonate-related osteonecrosis of the jaw in men with castrate-resistant prostate cancer. Ther Clin Risk Manag. 2010;6:579-83.

131. Balla B, Vaszilko M, Kósa JP, Podani J, Takács I, Tóbiás B, et al. New approach to analyze genetic and clinical data in bisphosphonate-induced osteonecrosis of the jaw. Oral Dis. 2012;18(6):580-5.

132. Such E, Cervera J, Terpos E, Bagán JV, Avaria A, Gómez I, et al. CYP2C8 gene polymorphism and bisphosphonate-related osteonecrosis of the jaw in patients with multiple myeloma. Haematologica. 2011;96(10):1557-9.

133. Arduino PG, Menegatti E, Scoletta M, Battaglio C, Mozzati M, Chiecchio A, et al. Vascular endothelial growth factor genetic polymorphisms and haplotypes in female patients with bisphosphonate-related osteonecrosis of the jaws. J Oral Pathol Med. 2011;40(6):510-5.

134. Choi H, Lee JH, Kim HJ, Park W, Lee JH, Kim JH. Genetic association between VEGF polymorphisms and BRONJ in the Korean population. Oral Dis. 2015;21(7):866-71.

135. Kim S, Mun S, Shin W, Han K, Kim MY. Identification of Potentially Pathogenic Variants Associated with Recurrence in Medication-Related Osteonecrosis of the Jaw (MRONJ) Patients Using Whole-Exome Sequencing. J Clin Med. 2022;11(8).
136. Aghaloo T, Hazboun R, Tetradis S. Pathophysiology of Osteonecrosis of the Jaws. Oral Maxillofac Surg Clin North Am. 2015;27(4):489-96.
137. Schiff MH. Role of interleukin 1 and interleukin 1 receptor antagonist in the mediation of rheumatoid arthritis. Ann Rheum Dis. 2000;59 Suppl 1(Suppl 1):i103-8.
138. Karimbux NY, Saraiya VM, Elangovan S, Allareddy V, Kinnunen T, Kornman KS, et al. Interleukin-1 gene polymorphisms and chronic periodontitis in adult whites: a systematic review and meta-analysis. J Periodontol. 2012;83(11):1407-19.
139. Schiodt M, Vadhan-Raj S, Chambers MS, Nicolatou-Galitis O, Politis C, Coropciuc R, et al. A multicenter case registry study on medication-related osteonecrosis of the jaw in patients with advanced cancer. Support Care Cancer. 2018;26(6):1905-15.
140. Wilde F, Heufelder M, Winter K, Hendricks J, Frerich B, Schramm A, et al. The role of surgical therapy in the management of intravenous bisphosphonates-related osteonecrosis of the jaw. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;111(2):153-63.
141. Vescovi P, Merigo E, Meleti M, Manfredi M, Guidotti R, Nammour S. Bisphosphonates-related osteonecrosis of the jaws: a concise review of the literature and a report of a single-centre experience with 151 patients. J Oral Pathol Med. 2012;41(3):214-21.
142. Ikesue H, Doi K, Morimoto M, Hirabatake M, Muroi N, Yamamoto S, et al. Risk evaluation of denosumab and zoledronic acid for medication-related osteonecrosis of the jaw in patients with bone metastases: a propensity score-matched analysis. Support Care Cancer. 2022;30(3):2341-8.
143. Hayashida S, Soutome S, Yanamoto S, Fujita S, Hasegawa T, Komori T, et al. Evaluation of the Treatment Strategies for Medication-Related Osteonecrosis of the Jaws (MRONJ) and the Factors Affecting Treatment Outcome: A Multicenter Retrospective Study with Propensity Score Matching Analysis. J Bone Miner Res. 2017;32(10):2022-9.
144. Ramaglia L, Guida A, Iorio-Siciliano V, Cuozzo A, Blasi A, Sculean A. Stage-specific therapeutic strategies of medication-related osteonecrosis of the jaws: a

systematic review and meta-analysis of the drug suspension protocol. Clin Oral Investig. 2018;22(2):597-615.

145. Petrovic M, Jelovac DB, Antic S, Antunovic M, Lukic N, Sabani M, et al. Medication-Related Osteonecrosis of the Jaws: Two Center Retrospective Cohort Studies. Biomed Res Int. 2019;2019:8345309.

146. Kang SH, Won YJ, Kim MK. Surgical treatment of stage 2 medication-related osteonecrosis of the jaw compared to osteomyelitis. Cranio. 2018;36(6):373-80.

147. Brodzikowska A, Górski B, Bogusławska-Kapała A. Effects of Interleukin-1 Genotype on the Clinical Efficacy of Non-Surgical Periodontal Treatment of Polish Patients with Periodontitis. Biomedicines. 2023;11(2).

148. Cardoso JM, Ribeiro AC, Palos C, Proença L, Noronha S, Alves RC. Association between IL-1A and IL-1B gene polymorphisms with peri-implantitis in a Portuguese population-a pilot study. PeerJ. 2022;10:e13729.

149. Wagner J, Kaminski WE, Aslanidis C, Moder D, Hiller KA, Christgau M, et al. Prevalence of OPG and IL-1 gene polymorphisms in chronic periodontitis. J Clin Periodontol. 2007;34(10):823-7.

150. Davis SM, Clark EA, Nelson LT, Silver RM. The association of innate immune response gene polymorphisms and puerperal group A streptococcal sepsis. Am J Obstet Gynecol. 2010;202(3):308.e1-8.

151. Johnson MD, Plantinga TS, van de Vosse E, Velez Edwards DR, Smith PB, Alexander BD, et al. Cytokine gene polymorphisms and the outcome of invasive candidiasis: a prospective cohort study. Clin Infect Dis. 2012;54(4):502-10.

152. Bagan J, Sheth CC, Soria JM, Margaix M, Bagan L. Bisphosphonates-related osteonecrosis of the jaws: a preliminary study of salivary interleukins. J Oral Pathol Med. 2013;42(5):405-8.

153. de Barros Silva PG, de Oliveira CC, Brizeno L, Wong D, Lima Júnior R, Gonçalves RP, et al. Immune cellular profile of bisphosphonate-related osteonecrosis of the jaw. Oral Dis. 2016;22(7):649-57.

154. Endo Y, Kumamoto H, Nakamura M, Sugawara S, Takano-Yamamoto T, Sasaki K, et al. Underlying Mechanisms and Therapeutic Strategies for Bisphosphonate-Related Osteonecrosis of the Jaw (BRONJ). Biol Pharm Bull. 2017;40(6):739-50.

155. Yamaguchi K, Motegi K, Iwakura Y, Endo Y. Involvement of interleukin-1 in the inflammatory actions of aminobisphosphonates in mice. Br J Pharmacol. 2000;130(7):1646-54.
156. Sugawara S, Shibazaki M, Takada H, Kosugi H, Endo Y. Contrasting effects of an aminobisphosphonate, a potent inhibitor of bone resorption, on lipopolysaccharide-induced production of interleukin-1 and tumour necrosis factor alpha in mice. Br J Pharmacol. 1998;125(4):735-40.
157. Deng X, Tamai R, Endo Y, Kiyoura Y. Alendronate augments interleukin-1beta release from macrophages infected with periodontal pathogenic bacteria through activation of caspase-1. Toxicol Appl Pharmacol. 2009;235(1):97-104.
158. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol. 2009;27:519-50.
159. Nakamura I, Jimi E. Regulation of osteoclast differentiation and function by interleukin-1. Vitam Horm. 2006;74:357-70.
160. Shih C, Huang MY. Interleukin-1 alpha stimulates osteoclast formation from peripheral blood monocytes and increases osteoclastic activity. Zhonghua Yi Xue Za Zhi (Taipei). 1996;57(2):85-92.
161. Muñoz-Salgado A, Silva FF, Padín-Iruegas ME, Camolesi GC, Bernaola-Paredes WE, Veronese HR, et al. Leukocyte and platelet rich fibrin in the management of medication-related osteonecrosis of the jaw: A systematic review and meta-analysis. Med Oral Patol Oral Cir Bucal. 2023;28(4):e317-e29.
162. Bracher AI, Vig N, Burkhard J-P, Schaller B, Schlittler F. The application of platelet rich fibrin in patients presenting with osteonecrosis of the jaw: A systematic literature review. Advances in Oral and Maxillofacial Surgery. 2021;2:100076.
163. Kim JW, Kim SJ, Kim MR. Leucocyte-rich and platelet-rich fibrin for the treatment of bisphosphonate-related osteonecrosis of the jaw: a prospective feasibility study. Br J Oral Maxillofac Surg. 2014;52(9):854-9.
164. Giudice A, Barone S, Bennardo F. Effect of Platelet-Rich Fibrin in Surgical Treatment of Medication-Related Osteonecrosis of the Jaw. J Oral Maxillofac Surg. 2020;78(10):1659.
165. Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest DM. The relevance of Choukroun's platelet-rich fibrin and metronidazole during complex maxillary

rehabilitations using bone allograft. Part I: a new grafting protocol. Implant Dent. 2009;18(2):102-11.
166. Dincă O, Zurac S, Stăniceanu F, Bucur MB, Bodnar DC, Vlădan C, et al. Clinical and histopathological studies using fibrin-rich plasma in the treatment of bisphosphonate-related osteonecrosis of the jaw. Rom J Morphol Embryol. 2014;55(3):961-4.
167. Gacche RN, Meshram RJ. Angiogenic factors as potential drug target: efficacy and limitations of anti-angiogenic therapy. Biochim Biophys Acta. 2014;1846(1):161-79.
168. Singh A, Kohli M, Gupta N. Platelet rich fibrin: a novel approach for osseous regeneration. J Maxillofac Oral Surg. 2012;11(4):430-4.

10. Bibliography of the candidate's publications

Publications related to the thesis:

- **Szentpeteri S**, Schmidt L, Restar L, Csaki G, Szabo G, Vaszilko M. The Effect of Platelet-Rich Fibrin Membrane in Surgical Therapy of Medication-Related Osteonecrosis of the Jaw. J Oral Maxillofac Surg. 2020 May;78(5):738-748. doi: 10.1016/j.joms.2019.12.008. Epub 2019 Dec 24. PMID: 31945309.
  IF:1,895 Q2
- **Szentpeteri S**, Kosa J, Juhasz DH, Deak Gy, Nemeth Zs, Lakatos P, Vaszilko M, Examination of certain single-nucleotide polymorphisms of interleukins 1A and 1B in medication-related osteonecrosis of the jaw — an ambirectional cohort study. J Cran Maxillofac Surg. 2024, In press, https://doi.org/10.1016/j.jcms.2024.06.007.
  Expected IF:2,1 D1/Q1

Publications not related to the thesis:

- **Szentpéteri S**, Horváth E, Dékány Sz, Krasznai M, Hornyák Cs, Tamás L. A szaglászcsökkenés vizsgálata neurodegeneratív megbetegedésben szenvedők körében (Examination of olfactory function in neugodegenerative disease). Otorhinolaryngologia Hungarica 2015; 61 (4):1 47-150.
- **Szentpéteri S**, Restár L, Németh Z, Vaszilkó M. A gyógyszer okozta állcsontnekrózis prognózisát befolyásoló faktorok [Prognostic factors of the medication-related osteonecrosis of the jaw]. Orv Hetil. 2020 Feb;161(8):283-289. Hungarian. doi: 10.1556/650.2019.31621. PMID: 32073291.
  IF:0,54, Q4
- Bojtor B, Vaszilko M, Armos R, Tobias B, Podani J, **Szentpeteri S**, Balla B, Lengyel B, Piko H, Illes A, Kiss A, Putz Z, Takacs I, Kosa JP, Lakatos P. Analysis of SIRT1 Gene SNPs and Clinical Characteristics in Medication-Related Osteonecrosis of the Jaw. Int J Mol Sci. 2024 Mar 25;25(7):3646. doi: 10.3390/ijms25073646. PMID: 38612458; PMCID: PMC11011248
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