applied sciences

MDPI

*Article*

# Assessment of Systemic and Maxillary Bone Loss in Cancer Patients with Endo-Periodontal Lesions Using Dkk-1 Biomarker and Dental Radiological Examinations

**Cristina Antohi [1], Mihaela Salceanu [1], Liana Aminov [1], Maria-Alexandra Martu [1,*], Cristina Gena Dascalu [2,*], Gianina Dodi [3], George Stoica [4], Geanina Bandol [5], Dragos Iancu [6], Bogdan Dobrovat [7] and Danisia Haba [7]**

1 Department of Odontontology, Periodontology and Fixed Restorations, Grigore T. Popa University of Medicine and Pharmacy of Iasi, 16 University Street, 700115 Iasi, Romania; cristina.antohi@umfiasi.ro (C.A.); mihaela.salceanu@umfiasi.ro (M.S.); liana.aminov@umfiasi.ro (L.A.)
2 Department of Medical Informatics and Biostatistics, Grigore T. Popa University of Medicine and Pharmacy of Iasi, 16 University Street, 700115 Iasi, Romania
3 Advanced Research and Development Center for Experimental Medicine, Grigore T. Popa University of Medicine and Pharmacy of Iasi, 16 University Street, 700115 Iasi, Romania; gianina.dodi@umfiasi.ro
4 Department of Dental Prosthetics, Dunarea de Jos University of Medicine and Pharmacy Galati, Domneasca Street 47, 800008 Galati, Romania; geostoica2003@gmail.com
5 Department of Ears Nose Throat (ENT), Regional Institute of Oncology, 2-4 General Henri Mathias Berthelot Street, 700483 Iasi, Romania; geanina-bandol@umfiasi.ro
6 Department of Radiotherapy, Regional Institute of Oncology, 2-4 General Henri Mathias Berthelot Street, 700483 Iasi, Romania; dragos.iancu@umfiasi.ro
7 Department of Radiology, Grigore T. Popa University of Medicine and Pharmacy of Iasi, 16 University Street, 700115 Iasi, Romania; bogdan-ionut.dobrovat@umfiasi.ro (B.D.); danisia.haba@umfiasi.ro (D.H.)
* Correspondence: maria-alexandra.martu@umfiasi.ro (M.-A.M.); cristina.dascalu@umfiasi.ro (C.G.D.); Tel.: +40-742-189-178 (M.-A.M.)

**Abstract:** The aim of our study was to correlate systemic bone loss by evaluating human Dickkopf-related protein 1 (Dkk-1) biomarker compared to horizontal bone loss as well as the presence and size of periapical lesions assessed by dental X-ray (ortopantomography—OPT) and cone beam computed tomography (CBCT) in patients with cancer in the ears, nose and throat (ENT) region vs. healthy controls. The study included 63 subjects divided into a study group of 33 cancer patients with ENT cancer (larynx/oropharynx/sinuses) and a control group of 30 healthy individuals. Blood samples were collected from both groups to assess Dkk-1 level using a sandwich enzyme immunoassay. The dental radiological examination consisted of a panoramic X-ray and a CBCT in order to appraise the horizontal bone loss, the presence and size of the periapical lesions in 2D vs. 3D images. The panoramic X-ray showed that in the control group, the maximum bone loss reached 13.2 mm, with an average of 4.930 $\pm$ 3.258 mm, while in the study group, the maximum horizontal bone loss was 11.3 mm, with an average of 5.191 $\pm$ 2.109 mm. The CBCT 3D investigation, when compared to the OPT, showed increased values for horizontal bone loss, both in the control group and in the study group; in the control group, the maximum bone loss reached 14.10 mm, with an average of 5.736 $\pm$ 3.471 mm, and in the study group, the maximum value was 12.40 mm, and the average was again slightly higher (6.152 $\pm$ 2.519 mm). The mean value for Dkk-1 in cancer patients was 1.209 $\pm$ 0.110 ng/mL, significantly lower than the value observed in healthy patients (1.712 $\pm$ 0.100 ng/mL). CBCT revealed higher values for the investigated parameters when compared to panoramic X-rays. Taking into account the preliminary nature of our study, we observed a significant correlation between the level of bone loss recorded by the Dkk-1 biomarker and radiological dental examination in patients with ENT cancer when compared to the control group.

**Keywords:** dental X-ray; CBCT; endo-perio lesion; bone loss; head and neck cancer; Dkk-1 expression

check for updates

**Citation:** Antohi, C.; Salceanu, M.; Aminov, L.; Martu, M.-A.; Dascalu, C.G.; Dodi, G.; Stoica, G.; Bandol, G.; Iancu, D.; Dobrovat, B.; et al. Assessment of Systemic and Maxillary Bone Loss in Cancer Patients with Endo-Periodontal Lesions Using Dkk-1 Biomarker and Dental Radiological Examinations. *Appl. Sci.* **2022**, *12*, 5235. https://doi.org/10.3390/app12105235

Academic Editor: Andrea Scribante

Received: 8 March 2022
Accepted: 20 May 2022
Published: 22 May 2022

## 1. Introduction

The incidence of cancer is increasing year by year. There is a need in the scientific community for early detection methods because by the time a clinical diagnosis is made, for the majority of patients, it is in a late stage, and thus the mortality rate is high. Research on markers related to prognosis of tumours is also increasing [1–3].

Human Dickkopf-related protein 1 (Dkk-1), composed of 266 amino acids, has a predicted molecular weight of 25.8 kDa, being a secretory protein from the *Dickkopf* gene family that comprises five evolutionarily conservative members [4]. Dkk-1 is a secretory antagonist of the classical Wnt signalling pathway. Dkk-1 was found to be abnormally expressed in tumour cells, inhibit cell proliferation and induce apoptosis through proapoptotic factors [5]. However, due to the differences in the tumour environment and the complex regulatory mechanisms in different tumours, Dkk-1 expresses differently in various types of tumours, such as lung, gastric, colorectal, pancreatic, bladder urothelial, breast, ovarian and cervical cancer, multiple myeloma, hepatocellular, oesophageal and oral squamous cell carcinoma [3,6–8]. Most of the reported studies point out that Dkk-1 plays a complex and different role in tumour occurrence, development and metastasis stimulation of various types of cancers or it is related to the late stage, all through a variety of complex regulatory mechanisms. More and more evidence has shown that Dkk-1 may not only be a useful biomarker of metastasis, but also a biological marker with the potential to evaluate tumour diagnosis and prognosis [6].

In oral-cavity-related diseases and conditions, Dkk-1 suppresses osteoblast differentiation but increases the expression of the osteoclast differentiation factor receptor activator of nuclear factor kappa B ligand (RANKL) and decreases the expression of osteoprotegerin (OPG), which induces osteoclastogenesis [9–11]. Dkk-1 is a key regulator of bone remodelling in physiological and pathological conditions, and blocking this factor may stimulate osteoblastogenesis and inhibit osteoclastogenesis [12–14]. Dkk-1 upregulation is associated with alveolar bone loss in humans with periodontal disease [15], but its inhibition is also related to the TNF-α inhibitor and an interleukin (IL)-1 receptor antagonist treatment in rheumatoid arthritis patients [16–19]. Therefore, a change in the level of Dkk-1 may serve as a biomarker of disease activity and bone erosion [20].

The above-mentioned OPG/ligand system, which is involved in osteoclast differentiation and activation, along with the Wnt pathway, also implicated in the regulation of bone homeostasis, can be considered the key routes regulating bone loss in several bone disorders, including osteoporosis, rheumatoid arthritis and periodontitis [21].

Periodontitis is chronic inflammation of the supportive apparatus of the tooth, and it is characterized by progressive loss of alveolar bone [22–24]. The defence mechanisms of the periodontal tissues lead to the initiation and progression of the inflammatory cascade that leads to bone resorption, which appears as radiolucency on radiographs around the root [25–28]. Apical periodontitis caused by endo-periodontal lesions can be symptomatic or asymptomatic and is generally observed through incidental findings during routine radiographic examinations using an orthopantomography (OPT) [29,30]. Although useful as a preliminary paraclinical examination, these techniques have significant limitations because of the inherent two-dimensional imaging of three-dimensional structures. In order to solve this drawback, cone beam computed tomography (CBCT) was developed [31]. Even though it offers superior clarity and details, due to the increased radiation exposure, the routine use of CBCT imaging in dental practices is not justified [31].

It is well-known that the immune response induced by periodontal disease is associated with an increase in inflammatory markers that are found in common chronic systemic diseases, for example, diabetes [32,33]. In a recent clinical study, the levels of oxidative stress in patients suffering from both diabetes and periodontal disease were assessed, and the results recommend these as an adjunctive marker of risk for the occurrence of complications in diabetic patients presenting periodontal disease [34]. Therefore, the authors demonstrated the relationship between diabetes and periodontal disease, a debated subject in the literature.

Additionally, there are numerous cross sectional and longitudinal studies that debate the association of periodontal disease and cancer risk; most suggest a positive association with overall cancer risk and certain specific types of cancer [35,36]. Regarding head and neck cancer, research evidence from recent years indicates that periodontal disease is linked to an increased risk of this type of cancer [36]. Clinical periodontal measures and/or proxy measures of periodontal disease have been used in evaluating its associations with head and neck cancers [36], with none evaluating the Dkk-1 biomarker. Therefore, there are insufficient data in the literature regarding bone loss caused by endo-periodontal lesions and the correlation with Dkk-1 values in healthy and oncologic patients.

In this context, the aim of our study was to correlate systemic bone loss by evaluating the Dkk-1 biomarker compared to horizontal bone loss as well as the presence and size of periapical lesions assessed by dental X-ray (OPT) and CBCT in patients with cancer in the ENT region vs. healthy controls.

## 2. Materials and Methods

### 2.1. Subjects

Thirty-three patients diagnosed with head and neck cancer by an ENT specialist through clinical, histopathological and CT examination (study group) and thirty healthy individuals were selected from the population referred to the "Regional Institute of Oncology" Iasi and "Grigore T. Popa" University of Medicine and Pharmacy of Iasi, from 1 June 2021 to 30 November 2021.

Subjects who fulfilled the following described inclusion/exclusion criteria were invited to enter this case–control study. All eligible subjects were informed of the purpose of the study, potential risks and benefits of their participation in the study and signed the informed consent before the start of the study. The ethics committee of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi previously approved this study protocol (no. 85/26.05.2021).

### 2.2. Inclusion and Exclusion Criteria

The inclusion criteria for the study group (cancer patients) were: histopathologically and CT-confirmed ENT cancer, aged between 24 and 80 years, no metastases, no radio/chemotherapy in the past or present and previous and current dental pathology.

Exclusion criteria for the group of oncological patients were: healthy patients, no dental diseases, patients with metastases, aged under 24 years and over 80 years, with radio/chemotherapy in the past and present.

For the control group, the inclusion criteria were: absence of any type of cancer, aged between 24 and 80 years and previous and current dental pathology.

Exclusion criteria for the control group were: the presence of any form of cancer, the presence of other systemic pathologies, antibiotic intake in the last 3 months, pregnant or lactating women, aged under 24 years and over 80 years and lack of dental diseases.

An ENT physician, two radiologists and two endodontists examined both groups.

### 2.3. Clinical Examinations

Our study was based on the correlation of horizontal bone loss from the dental level, measured between the coronal landmark (CL) cement–enamel junction and alveolar crest (AC) and the bone level (BL); the presence and size of periapical lesions were both involved in confirming the presence of dento-periodontal lesions leading to bone loss by using radiological examination (orthopantomography and CBCT). We measured the periapical lesions using the biggest diameter of lesion in any direction, using Romexis 4.4.2. For horizontal bone loss, we took into consideration the biggest length between CL and BL in any dental quadrant.

OPT parameters: Romexis 4.4.2, 70–74 KV, 10–12.5 mA, 15–16 s.

CBCT parameters: 85–90 KV, 8–12.5 mA, 13.7–16 s.

### 2.4. Serum Sampling

Peripheral blood samples were also collected in appropriate tubes (Serum BD Vacutainer) on the same day of the clinical examination. Immediately after coagulation, the serum was separated from blood by centrifugation (10 min at 4500 rpm) and stored in aliquots in 0.5 mL Eppendorf tubes at −80 °C for subsequent enzyme-linked immunosorbent assay (ELISA) analysis. The tubes were marked with a specific ID depending on the batch they belonged to: control (M) or study (S).

### 2.5. ELISA

The level of DKK-1 (Lot no. E-16-023P04S01, BioVendor–Laboratorni medicina a.s, Brno, Czech Republic) in the serum was evaluated by ELISA assay according to the manufacturer's recommendations. Briefly, 100 µL of standards, blank and samples were added into appropriate wells in duplicate and incubated at room temperature for 1 h with shaking. The plates were then washed 5 times (using an automated microplate washer, model Tecan—Hydroflex platform, Grödig, Austria), and 100 µL of biotin-labelled antibody was added to all wells. After 1 h at room temperature, the plates were washed again and incubated with 100 µL of streptavidin-HRP conjugate for 30 min. Subsequently, the plate was washed 5 times again, and 100 µL of substrate was added and incubated for 10 min at room temperature in the dark (aluminium foil). The reaction was stopped by the addition of 100 µL of stop solution. The colour was measured using a microplate reader at 450 nm with the reference wavelength set to 570 nm (Microplate Reader/Model Sunrise Basic Tecan, Grödig, Austria).

Results were calculated using the standard curve constructed by plotting the absorbance of standards against the known concentrations of standards (0.125–4 ng/mL) in logarithmic scale, using the four-parameter algorithm. The level of Dkk-1 was expressed as nanograms per millilitre of serum (ng/mL).

### 2.6. Statistical Analysis

The statistical analysis was conducted in SPSS 27.0 and Statistica 14.0. Continuous data were characterized by averages, standard deviations and standard errors, and categorical data were expressed as percentages. We performed univariate data analysis using the chi-square and Student's $t$ tests. All tests were 2-tailed; a $p$ value $\leq 0.05$ was considered statistically significant.

## 3. Results and Discussions

Table 1 presents the demographic characteristics of the study population for both groups. As observed, there was no significant difference in the mean age between groups ($p > 0.05$), but there was an unbalanced gender distribution in the study group.

**Table 1.** Demographic characteristics of the study population (mean ± SD = standard deviation).

| | **Total ($n$ = 63)** | **Control ($n$ = 30)** | **ENT Cancer ($n$ = 33)** | **$p$ Value** |
|---|---|---|---|---|
| Gender: $n$ (%) | | | | |
| - Male | 38 (60.3) | 15 (50.0) | 23 (69.7) | 0.110 |
| - Female | 25 (39.7) | 15 (50.0) | 10 (30.3) | |
| Age: m ± SD | | | | |
| - Total | 52.67 ± 12.241 | 53.87 ± 10.760 | 51.58 ± 13.521 | 0.463 |
| - Male | 52.84 ± 12.985 | 54.93 ± 12.567 | 51.48 ± 13.348 | |
| - Female | 52.40 ± 11.269 | 52.80 ± 8.914 | 51.80 ± 14.642 | |
| Cancer type: $n$ (%) | | | | |
| - Larynx | | | 24 (72.7) | |
| - Oropharynx | | | 4 (12.1) | |
| - Sinuses | | | 5 (15.2) | |

**Table 1.** *Cont.*

| | Total ($n$ = 63) | Control ($n$ = 30) | ENT Cancer ($n$ = 33) | $p$ Value |
|---|---|---|---|---|
| Oral mucositis degree: $n$ (%) | | | | |
| - III | | | 20 (60.6) | |
| - IV | | | 13 (39.4) | |
| Irradiation degree: $n$ (%) | | | | |
| - 66 Gy/33 fr | | | 13 (39.4) | |
| - 70 Gy/35 fr | | | 20 (60.6) | |

The OPT investigation showed that in the control group, the maximum bone loss reached 13.2 mm, with an average of 4.930 ± 3.258 mm, while in the study group, the maximum horizontal bone loss was 11.3 mm, with an average of 5.191 ± 2.109 mm. The CBCT 3D investigation displayed increased values for horizontal bone loss, both in the control and in the study group; in controls, the maximum horizontal bone loss reached 14.10 mm, with an average of 5.736 ± 3.471, and in the study group, the maximum value was 12.40 mm, and the average was again slightly higher (6.152 ± 2.519) (Table 2) (Figures 1–3).

**Table 2.** Horizontal bone loss: comparative descriptive statistics in the control/ENT cancer group.

| Horizontal Bone Loss—Max Size | Group | Number | Mean | Standard Average Error | SD | Min | Max | Median | Student's $t$ Test |
|---|---|---|---|---|---|---|---|---|---|
| OPT | Control | 30 | 4.930 | 0.594 | 3.258 | 0.0 | 13.2 | 4.60 | $t$ = −0.373 |
| | ENT Cancer | 33 | 5.191 | 0.367 | 2.109 | 0.0 | 11.3 | 5.00 | $p$ = 0.711 |
| | Total | 63 | 5.067 | 0.340 | 2.698 | 0.0 | 13.2 | 4.80 | |
| CBCT 3D | Control | 30 | 5.736 | 0.633 | 3.471 | 0.0 | 14.10 | 5.75 | $t$ = −0.548 |
| | ENT Cancer | 33 | 6.152 | 0.438 | 2.519 | 0.0 | 12.40 | 6.17 | $p$ = 0.586 |
| | Total | 63 | 5.954 | 0.377 | 2.992 | 0.0 | 14.10 | 6.00 | |

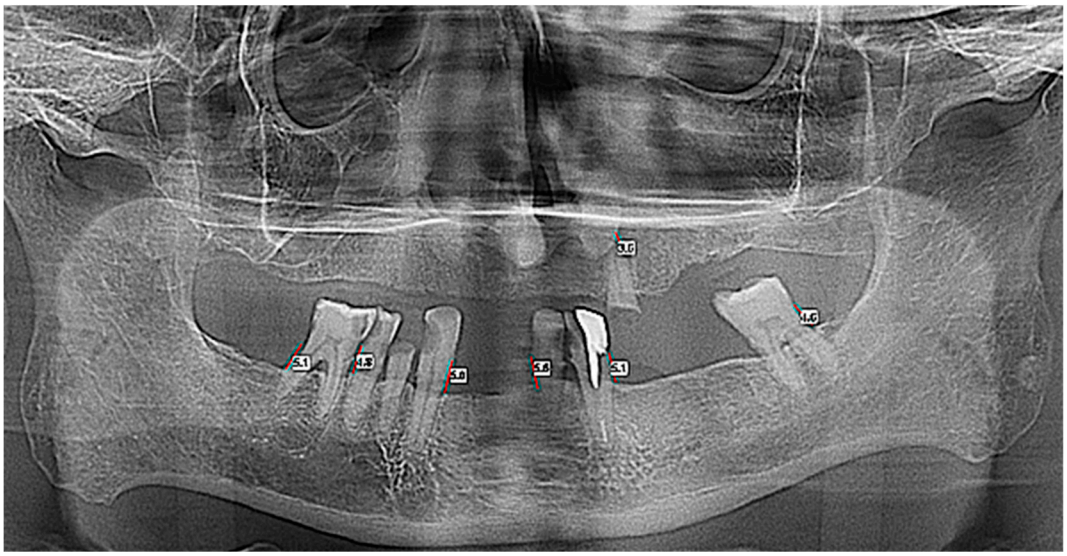

**Figure 1.** Orthopantomography of patient A.D., larynx cancer, 62 years old, assessment of horizontal bone loss (maximum 5.1 mm at tooth 46) and measurement of the size of periapical lesions present at tooth 23 is 3.6 mm.

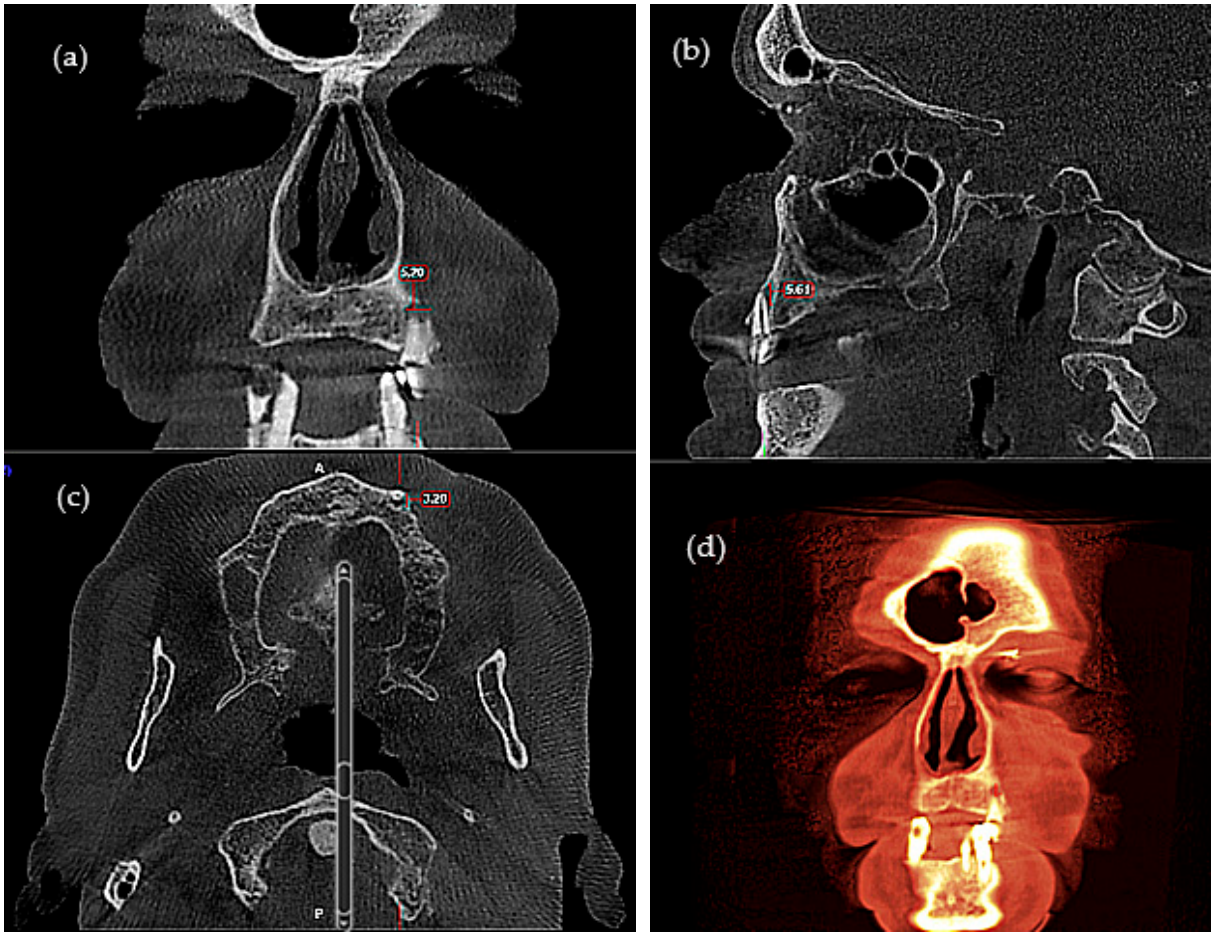


**Figure 2.** Patient A.D., larynx cancer, 62 years old. (**a**) Frontal CBCT cross section of the periapical lesion in tooth 23 is 5.2 mm; (**b**) sagittal CBCT cross section of the periapical lesion in tooth 23 is 5.61 mm; (**c**) coronal CBCT cross section of the periapical lesion in tooth 23 is 3.2 mm; (**d**) 3D CBCT cross section of the periapical lesion in tooth 23.

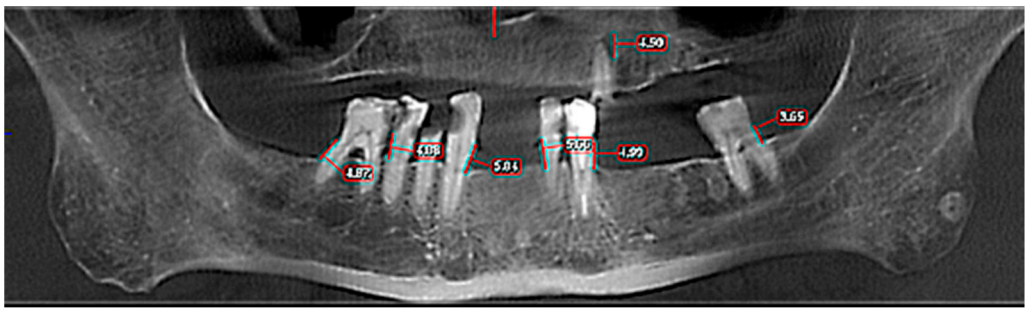


**Figure 3.** OPT–CBCT of Patient A.D., assessment of horizontal bone loss (maximum 6.8 mm at tooth 46) and measurement of the size of periapical lesions present at 23 is 4.5 mm.

When evaluating the OPT, the dimensions of the periapical lesions found in the control group varied between 0 and 5.00 mm vs. 0 and 6.00 mm in ENT cancer patients. The evaluation of CBCT 3D highlights the same phenomenon, but the magnitude of the periapical lesions found was higher, both in the control group and in the study group, namely, 7.60 mm for the group control and 10.81 mm for ENT cancer.

The comparative study of the measurements performed on CBCT 3D and OPT at the level of each of the two groups showed that the discrepancy between the two methods, both in terms of depth of lesions and in terms of the extent of horizontal bone loss, was statistically significant. The CBCT 3D investigation always revealed higher values for the investigated parameters than the OPT investigation. As such, CBCT allowed us to assess bone loss in relation to adjacent anatomical structures, which plays an important role in treatment, both periodontal and endodontic, as well as in the insertion of dental implants evaluated by paraxial CBCT cross sections. Coronal, sagittal and axial CBCT cross sections allowed us to evaluate the periapical lesions in a 3D format, which plays an important role in their treatment plan for preserving the teeth with this pathology, or otherwise, their extraction if there is such a recommendation.

As previously stated, one of the side effects of radiation therapy is osteoradionecrosis [37]. Extensive periapical lesions or severe periodontal disease are a favourable factor for the development of this pathology, which is why we performed an OPT and CBCT radiological examination to evaluate the size of the lesions in the 3D version and to select the teeth that could be preserved for conservative therapy and those that needed to be extracted to avoid osteoradionecrosis after radiotherapy. Muller et al. [14] observed that bone level gradually decreased with each decade, and from 50 years and older, the bone loss was even more pronounced, reaching a mean of about 1.8 mm at that age. Moreover, infrabony defects were more pronounced in the maxilla, especially at the molar level [14]. A study examined the differences in bone density before and after endodontic treatment in teeth with periapical lesions, and the authors concluded that the use of CBCT to measure bone density before and after endodontic treatment is recommended for teeth with periapical lesions [38].

Alternatively, in a recent study, Nardi et al. [39] revealed that periapical lesions can be detected with similar accuracy on OPT vs. CBCT except those that are less than 4.5 mm in size and those that are located in the upper and lower incisor areas. On the other hand, Antony et al. [40] observed that CBCT had higher accuracy in the detection of periapical lesions compared to periapical and panoramic radiography.

As stated in the Introduction, quantifying disease biomarkers is of great importance for early interventions to monitor disease progression or to evaluate treatment responses [41,42]. Moreover, one should not underestimate the importance of age as a modifier of disease progression and treatment [43]. Besides its role as a potential biomarker for cancer progression and prognosis, Dkk-1 can be also considered an efficacious treatment strategy for preventing cancer metastasis in oral squamous cell carcinoma and also can be a molecular marker for early detection of lymph node metastasis [2,3].

Regarding the comparative evaluations of the results obtained by the ELISA assay in the control patients compared to those with cancer, there were statistically significant differences. The mean value for Dkk-1 in cancer patients was $1.209 \pm 0.110$ ng/mL, significantly lower than the similar value observed in healthy patients of $1.712 \pm 0.100$ ng/mL (Figure 4). The obtained results could represent the different outcomes of both cancer and periodontal disease, since it is well-known that Dkk-1 is a critical secreted factor that modulates the microenvironment [44].

In terms of cancer, the strong duality of the role of Dkk-1 is remarkable [45]. In many tumours, Dkk-1 clearly acts only as an oncogene, but in others, it is found to have an anti-oncogenic role, depending on the setting complexity and/or the given tumour type.

It was reported that serum Dkk-1 in patients with osteonecrosis of the femoral head could be correlated with the severity of this pathology. Moreover, increased Dkk-1 expression was associated with bone cell apoptosis in patients with corticosteroid and alcohol intake and progression of osteonecrosis of the femoral head [46]. Researchers stated that the Dkk-1 expression level at clinical stage I in endometrial carcinoma was significantly greater than at other stages [47]. Hall et al. [48] also observed that Dkk-1 levels appeared faster in an early diagnosis of prostate cancer compared to the late stages with bone metastases,

suggesting that Dkk-1 expression increases in early-stage cancer and is a necessary early event to support osteolysis.

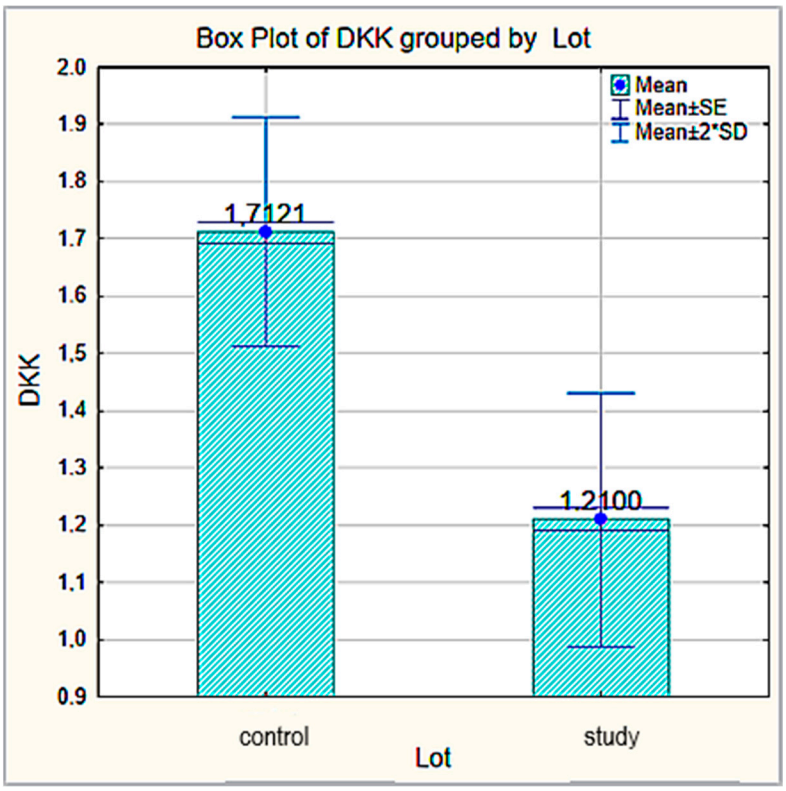


**Figure 4.** Dkk-1: comparative descriptive statistics in the control/test group.

As we well know, Dkk-1 has been associated with multiple myeloma and various types of cancers, including head and neck, lung, breast, liver and bone cancers [3,6]. Mazon et al. discovered that patients with acute infections showed dramatically high levels of Dkk-1 (6072 ± 518 pg/mL) in their blood compared to healthy blood donors (1726 ± 95 pg/mL) [4,49]. On the other hand, other researchers found higher Dkk-1 protein levels in myeloma cells from patients with bone lesions when compared to plasma cells from non-myeloma subjects or in plasma cells from patients who had myeloma without bone lesions [50].

A recent study observed that increased levels of serum Dkk-1 were noted in subjects with oesophageal adenocarcinoma, implying that this molecule has a crucial role in inflammation. Additionally, increased Dkk-1 values were associated with unfavourable five-year survival rates and the presence of circulating tumour cells [51]. The same authors noted that significantly lower levels were observed in subjects after neoadjuvant treatment, implying that Dkk-1 may act as a biomarker for treatment monitoring [51]. However, it is not always clear, since a plethora of examples for both sides may be found.

Coming back to the obtained results, a question is raised: could the decreased levels of Dkk-1 be bone-loss-protective when observed in low levels in the serum of cancer patients? The obtained low levels could be the effect of the cancer treatment and/or other chronic events in association with periodontal variables. Periodontal disease induces a number of oral environment modifications, and one must not minimize the role of the oral microbiome in the overall systemic ecological balance [52,53]. More studies are needed in order to suggest an association between bone markers such as the Dkk-1 and progression of radiological damage in ENT cancer subjects.

The limitations of our study consist of a small number of analyzed subjects, and a slight imbalance in the females-to-males ratio, which could affect the results. However, this

is explained by the reduced compliance and willingness of cancer patients to participate in research due to the altered quality of life. Another limitation is the fact that we do not have the follow-up values that would show the variation in time of bone loss and Dkk-1 values according to treatment.

## 4. Conclusions

CBCT provides crucial information regarding the diagnosis and treatment plan for periapical lesions. The assessment of horizontal bone loss by CBCT was clearly superior to that of OPT. CBCT should not be performed routinely, but in accordance with the ALARA (as low as possible) principle. Taking into account the preliminary nature of our study, we observed a significant correlation between the level of bone loss recorded by the Dkk-1 biomarker and radiological dental examinations in patients with ENT cancer when compared to the control group.

Regarding the bone loss evaluated systemically by the biomarker Dkk-1, a statistically significant correlation was observed with dental bone loss in both analyzed groups of ENT cancer and systemically healthy patients.

**Author Contributions:** Conceptualization, C.A. and D.H.; methodology, D.I. and M.S.; validation, C.G.D. and M.-A.M.; formal analysis, C.G.D. and G.D.; investigation, M.S., L.A. and G.B.; resources, B.D.; data curation, D.I. and G.S.; writing—original draft preparation, C.A. and G.D.; writing—review and editing, M.-A.M. and B.D.; visualization, L.A. and G.S.; supervision, D.H.; project administration, D.H.; funding acquisition, D.H. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Grigore T. Popa University of medicine and Pharmacy of Iasi (protocol code nr. 85/26.05.2021).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The data used to support the findings of this study are available from the corresponding author upon reasonable request.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Hristova, V.A.; Chan, D.W. Cancer biomarker discovery and translation: Proteomics and beyond. *Expert Rev. Proteom.* **2019**, *16*, 93–103. [CrossRef] [PubMed]
2. Huang, J.; Lu, T.; Kuang, W. Prognostic role of dickkopf-1 in patients with cancer. *Medicine* **2020**, *99*, e20388. [CrossRef] [PubMed]
3. Li, J.; Gao, Y.; Yue, W. The Clinical Diagnostic and Prognostic Value of Dickkopf-1 in Cancer. *Cancer Manag. Res.* **2020**, *12*, 4253–4260. [CrossRef] [PubMed]
4. Mazon, M.; Larouche, V.; St-Louis, M.; Schindler, D.; Carreau, M. Elevated blood levels of Dickkopf-1 are associated with acute infections. *Immun. Inflamm. Dis.* **2018**, *6*, 428–434. [CrossRef] [PubMed]
5. Zhu, G.; Song, J.; Chen, W.; Yuan, D.; Wang, W.; Chen, X.; Liu, H.; Su, H.; Zhu, J. Expression and Role of Dickkopf-1 (Dkk1) in Tumors: From the Cells to the Patients. *Cancer Manag. Res.* **2021**, *13*, 659–675. [CrossRef]
6. Tung, E.K.-K.; Mak, C.K.-M.; Fatima, S.; Lo, R.C.-L.; Zhao, H.; Zhang, C.; Dai, H.; Poon, R.T.-P.; Yuen, M.-F.; Lai, C.-L.; et al. Clinicopathological and prognostic significance of serum and tissue Dickkopf-1 levels in human hepatocellular carcinoma. *Liver Int.* **2011**, *31*, 1494–1504. [CrossRef]
7. Jaschke, N.; Hofbauer, L.C.; Göbel, A.; Rachner, T.D. Evolving functions of Dickkopf-1 in cancer and immunity. *Cancer Lett.* **2020**, *482*, 1–7. [CrossRef]
8. Feng, Y.; Zhang, Y.; Wei, X.; Zhang, Q. Correlations of DKK1 with pathogenesis and prognosis of human multiple myeloma. *Cancer Biomark.* **2019**, *24*, 195–201. [CrossRef]
9. Tan, X.; Huang, D.; Zhou, W.; Yan, L.; Yue, J.; Lu, W.; Song, D.; Zhou, X.; Ye, L.; Zhang, L. Dickkopf-1 may regulate bone coupling by attenuating wnt/β-catenin signaling in chronic apical periodontitis. *Arch. Oral Biol.* **2018**, *86*, 94–100. [CrossRef]
10. Pinzone, J.J.; Hall, B.M.; Thudi, N.K.; Vonau, M.; Qiang, Y.W.; Rosol, T.J.; Shaughnessy, J.D., Jr. The role of Dickkopf-1 in bone development, homeostasis, and disease. *Blood J. Am. Soc. Hematol.* **2009**, *113*, 517–525. [CrossRef]
11. Huang, Y.; Liu, L.; Liu, A. Dickkopf-1: Current knowledge and related diseases. *Life Sci.* **2018**, *209*, 249–254. [CrossRef] [PubMed]

12. Kong, Q.; Liu, L.; Huang, Y.; Zhang, F.; Wei, X.; Ling, J. The effect of octamer-binding transcription factor 4B1 on microRNA signals in human dental pulp cells with inflammatory response. *J. Endod.* **2014**, *40*, 101–108. [CrossRef] [PubMed]
13. He, W.; Wang, Z.; Zhou, Z.; Zhang, Y.; Zhu, Q.; Wei, K.; Lin, Y.; Cooper, P.; Smith, A.J.; Yu, Q. Lipopolysaccharide enhances Wnt5a expression through toll-like receptor 4, myeloid differentiating factor 88, phosphatidylinositol 3-OH kinase/AKT and nuclear factor kappa B pathways in human dental pulp stem cells. *J. Endod.* **2014**, *40*, 69–75. [CrossRef] [PubMed]
14. Müller, H.P.; Ulbrich, M.; Heinecke, A. Alveolar bone loss in adults as assessed on panoramic radiographs.(II) Multilevel models. *Clin. Oral Investig.* **2005**, *9*, 105–110. [CrossRef]
15. Wang, S.Y.; Liu, Y.Y.; Ye, H.; Guo, J.P.; Li, R.; Liu, X.; Li, Z.G. Circulating Dickkopf-1 is correlated with bone erosion and inflammation in rheumatoid arthritis. *J. Rheumatol.* **2011**, *38*, 821–827. [CrossRef]
16. Hartung, N.; Benary, U.; Wolf, J.; Kofahl, B. Paracrine and autocrine regulation of gene expression by Wnt-inhibitor Dickkopf in wild-type and mutant hepatocytes. *BMC Syst. Biol.* **2017**, *11*, 98. [CrossRef]
17. Fujita, K.; Janz, S. Attenuation of WNT signaling by DKK-1 and -2 regulates BMP2-induced osteoblast differentiation and expression of OPG, RANKL and M-CSF. *Mol. Cancer* **2007**, *6*, 71. [CrossRef]
18. Martu, A.; Rezus, E.; Sufaru, I.; Banu, C.; Martu, S.; Foia, L. Study on the clinical changes in general and oral status in patients with rheumatoid arthritis. *Rom. J. Oral Rehabil.* **2018**, *10*, 188–198.
19. Martu, M.A.; Solomon, S.M.; Toma, V.; Maftei, G.A.; Iovan, A.; Gamen, A.; Hurjui, L.; Rezus, E.; Foia, L.; Forna, N.C. The importance of cytokines in periodontal disease and rheumatoid arthritis. Review. *Rom. J. Oral Rehabil.* **2019**, *11*, 220–240.
20. Crotti, T.; Smith, M.D.; Hirsch, R.; Soukoulis, S.; Weedon, H.; Capone, M.; Ahern, M.J.; Haynes, D. Receptor activator NF kappaB ligand (RANKL) and osteoprotegerin (OPG) protein expression in periodontitis. *J. Periodontal Res.* **2003**, *38*, 380–387. [CrossRef]
21. Niehrs, C. Function and biological roles of the Dickkopf family of Wnt modulators. *Oncogene* **2006**, *25*, 7469–7481. [CrossRef] [PubMed]
22. Maithri, M.; Ballal, D.G.; Kumar, S.; Raghavendra, U.; Gudigar, A.; Chan, W.Y.; Macherla, S.; Vineetha, R.; Gopalkrishna, P.; Ciaccio, E.J.; et al. Development of a Computational Tool for the Estimation of Alveolar Bone Loss in Oral Radiographic Images. *Computation* **2022**, *10*, 8. [CrossRef]
23. Mocanu, R.C.; Martu, M.-A.; Luchian, I.; Sufaru, I.G.; Maftei, G.A.; Ioanid, N.; Martu, S.; Tatarciuc, M. Microbiologic Profiles of Patients with Dental Prosthetic Treatment and Periodontitis before and after Photoactivation Therapy—Randomized Clinical Trial. *Microorganisms* **2021**, *9*, 713. [CrossRef] [PubMed]
24. Pick, E.; Schäfer, T.; Al-Haj Husain, A.; Rupp, N.J.; Hingsammer, L.; Valdec, S. Clinical, Radiological, and Pathological Diagnosis of Fibro-Osseous Lesions of the Oral and Maxillofacial Region: A Retrospective Study. *Diagnostics* **2022**, *12*, 238. [CrossRef] [PubMed]
25. Zaharescu, A.; Solomon, S.M.; Luca, M.G.; Toma, V.; Luchian, I.; Sufaru, I.G.; Martu, M.A.; Foia, L.; Martu, S. Quantification of proinflammatory molecules (IL1-α, IL1-β, IL2, IL12, IFN-γ, TNF-α) in crevicular fluid and serum in patients with endo-periodontal lesions. *Rev. Chim.* **2019**, *70*, 2252–2255. [CrossRef]
26. Cohen, R.S.; Goldberger, T.; Merzlak, I.; Tsesis, I.; Chaushu, G.; Avishai, G.; Rosen, E. The Development of Large Radicular Cysts in Endodontically Versus Non-Endodontically Treated Maxillary Teeth. *Medicina* **2021**, *57*, 991. [CrossRef]
27. Pinto, D.; Marques, A.; Pereira, J.F.; Palma, P.J.; Santos, J.M. Long-Term Prognosis of Endodontic Microsurgery—A Systematic Review and Meta-Analysis. *Medicina* **2020**, *56*, 447. [CrossRef]
28. Galler, K.M.; Weber, M.; Korkmaz, Y.; Widbiller, M.; Feuerer, M. Inflammatory Response Mechanisms of the Dentine–Pulp Complex and the Periapical Tissues. *Int. J. Mol. Sci.* **2021**, *22*, 1480. [CrossRef]
29. Tang, Z.; Liu, X.; Chen, K. Comparison of digital panoramic radiography versus cone beam computerized tomography for measuring alveolar bone. *Head Face Med.* **2017**, *13*, 2. [CrossRef]
30. Tchorz, J.P.; Wrbas, K.T.; Von See, C.; Vach, K.; Patzelt, S.B.M. Accuracy of software-based three-dimensional root canal length measurements using cone-beam computed tomography. *Eur. Endod. J.* **2019**, *4*, 28–32. [CrossRef]
31. Kruse, C.; Spin-Neto, R.; Wenzel, A.; Kirkevang, L.L. Cone beam computed tomography and periapical lesions: A systematic review analysing studies on diagnostic efficacy by a hierarchical model. *Int. Endod. J.* **2015**, *48*, 815–828. [CrossRef] [PubMed]
32. Nesse, W.; Linde, A.; Abbas, F.; Spijkervet, F.K.; Dijkstra, P.U.; de Brabander, E.C.; Gerstenbluth, I.; Vissink, A. Dose-response relationship between periodontal inflamed surface area and HbA1c in type 2 diabetics. *J. Clin. Periodontol.* **2009**, *36*, 295–300. [CrossRef] [PubMed]
33. Maftei, G.-A.; Martu, M.-A.; Martu, M.-C.; Popescu, D.; Surlin, P.; Tatarciuc, D.; Popa, C.; Foia, L.-G. Correlations between Salivary Immuno-Biochemical Markers and HbA1c in Type 2 Diabetes Subjects before and after Dental Extraction. *Antioxidants* **2021**, *10*, 1741. [CrossRef] [PubMed]
34. Marconcini, S.; Giammarinaro, E.; Cosola, S.; Oldoini, G.; Genovesi, A.; Covani, U. Effects of Non-Surgical Periodontal Treatment on Reactive Oxygen Metabolites and Glycemic Control in Diabetic Patients with Chronic Periodontitis. *Antioxidants* **2021**, *10*, 1056. [CrossRef]
35. Kappenberg-Niţescu, D.C.; Luchian, I.; Mârțu, I.; Solomon, S.M.; Mârţu, S.; Păsărin, L.; Mârțu, A.; Sioustis, I.A.; Goriuc, A.; Tatarciuc, M. Periodontal effects of two innovative oral rinsing substances in oncologic patients. *Exp. Ther. Med.* **2021**, *21*, 98. [CrossRef]
36. Nwizu, N.; Wactawski-Wende, J.; Genco, R.J. Periodontal disease and cancer: Epidemiologic studies and possible mechanisms. *Periodontology* **2020**, *83*, 213–233. [CrossRef]

37. De Felice, F.; Tombolini, V.; Musio, D.; Polimeni, A. Radiation therapy and mandibular osteoradionecrosis: State of the art. *Curr. Oncol. Rep.* **2020**, *22*, 89. [CrossRef]
38. Kaya, S.; Yavuz, İ.; Uysal, İ.; Akkuş, Z. Measuring bone density in healing periapical lesions by using cone beam computed tomography: A clinical investigation. *J. Endod.* **2012**, *38*, 28–31. [CrossRef]
39. Nardi, C.; Calistri, L.; Pradella, S.; Desideri, I.; Lorini, C.; Colagrande, S. Accuracy of orthopantomography for apical periodontitis without endodontic treatment. *J. Endod.* **2017**, *43*, 1640–1646. [CrossRef]
40. Antony, D.P.; Thomas, T.; Nivedhitha, M.S. Two-dimensional periapical, panoramic radiography versus three-dimensional cone-beam computed tomography in the detection of periapical lesion after endodontic treatment: A systematic review. *Cureus* **2020**, *12*, e7736. [CrossRef]
41. Martu, M.A.; Maftei, G.A.; Luchian, I.; Popa, C.; Filioreanu, A.M.; Tatarciuc, D.; Nichitean, G.; Hurjui, L.-L.; Foia, L.-G. Wound healing of periodontal and oral tissues: Part II—Patho-phisiological conditions and metabolic diseases. *Rom. J. Oral Rehabil.* **2020**, *12*, 30–40.
42. Martu, M.A.; Maftei, G.A.; Sufaru, I.G.; Jelihovschi, I.; Luchian, I.; Hurjui, L.; Martu, I.; Pasarin, L. COVID-19 and periodontal disease: Etiopathogenic and clinical immplications. *Rom. J. Oral Rehabil.* **2020**, *12*, 116–124.
43. Popa, C.; Filioreanu, A.M.; Stelea, C.; Alexandru Maftei, G.A.; Popescu, E. Prevalence of oral lesions modulated by patients age: The young versus the elderly. *Rom. J. Oral Rehabil.* **2018**, *10*, 50–56.
44. Menezes, M.E.; Devine, D.J.; Shevde, L.A.; Samant, R.S. Dickkopf1: A tumor suppressor or metastasis promoter? *Int. J. Cancer* **2012**, *130*, 1477–1483. [CrossRef]
45. Giralt, I.; Gallo-Oller, G.; Navarro, N.; Zarzosa, P.; Pons, G.; Magdaleno, A.; Segura, M.F.; de Toledo, J.S.; Moreno, L.; Gallego, S.; et al. Dickkopf Proteins and Their Role in Cancer: A Family of Wnt Antagonists with a Dual Role. *Pharmaceuticals* **2021**, *14*, 810. [CrossRef]
46. Ko, J.Y.; Wang, F.S.; Wang, C.J.; Wong, T.; Chou, W.Y.; Tseng, S.L. Increased Dickkopf-1 expression accelerates bone cell apoptosis in femoral head osteonecrosis. *Bone* **2010**, *46*, 584–591. [CrossRef]
47. Yi, N.; Liao, Q.P.; Li, T.; Xiong, Y. Novel expression profiles and invasiveness-related biology function of DKK1 in endometrial carcinoma. *Oncol. Rep.* **2009**, *21*, 1421–1427.
48. Hall, C.L.; Daignault, S.D.; Shah, R.B.; Pienta, K.J.; Keller, E.T. Dickkopf-1 expression increases early in prostate cancer development and decreases during progression from primary tumor to metastasis. *Prostate* **2008**, *68*, 1396–1404. [CrossRef]
49. Mazon, M.; Masi, D.; Carreau, M. Modulating Dickkopf-1: A Strategy to Monitor or Treat Cancer? *Cancers* **2016**, *8*, 62. [CrossRef]
50. Tian, E.; Zhan, F.; Walker, R.; Rasmussen, E.; Ma, Y.; Barlogie, B.; Shaughnessy, J.D., Jr. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N. Engl. J. Med.* **2003**, *349*, 2483–2494. [CrossRef]
51. Ramirez, J.G.; Smit, D.J.; Viol, F.; Schrader, J.; Ghadban, T.; Pantel, K.; Izbicki, J.R.; Reeh, M. High Serum Levels of Wnt Signaling Antagonist Dickkopf-Related Protein 1 Are Associated with Impaired Overall Survival and Recurrence in Esophageal Cancer Patients. *Cancers* **2021**, *13*, 4980. [CrossRef] [PubMed]
52. Lee, Y.-H.; Chung, S.W.; Auh, Q.-S.; Hong, S.-J.; Lee, Y.-A.; Jung, J.; Lee, G.-J.; Park, H.J.; Shin, S.-I.; Hong, J.-Y. Progress in Oral Microbiome Related to Oral and Systemic Diseases: An Update. *Diagnostics* **2021**, *11*, 1283. [CrossRef] [PubMed]
53. Maftei, G.A.; Martu, C.M.; Popa, C.; Geletu, G.; Danila, V.; Jelihovschi, I.; Foia, L. The biomechanical properties of suture materials and their relationship to bacterial adherence. *Mater. Plast.* **2019**, *56*, 980–985. [CrossRef]