

Supplementary material

European Journal of Clinical Microbiology & Infectious Diseases

***Actinomyces* lesions and acute inflammation predominate in osteonecrosis of the jaw associated with osteoclast-suppressing therapy in contrast to non-medication-related osteonecrosis**

Authors

Marjo Kivelä-Rajamäki*, Hanna Välimaa, Jussi Furuholm, Caj Haglund, Timo Sorsa, Jaana Hagström, Asko Järvinen

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Corresponding author: Marjo Kivelä-Rajamäki Department of Infectious Diseases, Finland e-mail: marjo.kivela@helsinki.fi, marjo.kivela-rajamaki@hus.fi Address: HUS, Inflammation Centre, Division of Infectious Diseases, P.O. Box 340, 00029 HUS, Helsinki, Finland, Phone: +358-40-7351768 <https://orcid.org/0009-0004-1245-3071>

Supplementary Table 1. Immunohistochemistry protocol for antibodies.

Anti-body	Manu- facturer	Dilution ¹⁾	Pretreatment time (min)	Secondary Ab ²⁾ time (min)	DAB Chromogen ³⁾	Pretreatment pH	Incubation time
MMP-8	Prikk et al 2002 ^{a)}	1:300	15	20	AEC	9	ON - 4°C
TIMP-1	R&D AF970 ^{b)}	1:150	10	30 GOAT	AEC Rom	9	ON - 4°C

a) non-commercial antibody, protocol according to the authors [29]. b) R&D systems AF970, Bio-Tec, Minneapolis, MN, USA) polyclonal antibody. 1) Dako REAL Antibody Diluent S2022, 2) HRP labelled polymer secondary antibody (EnVision Flex/HRP SM802), 3) EnVision Flex DAB DM827, ON Counterstain (Dako Mayer's Haematoxylin S3309).

Abbreviations: DAB, diaminobenzidine; MMP-8, matrix metalloproteinase-8; ON, overnight; TIMP-1 tissue inhibitor of metalloproteinase-1.

Supplementary Table 2. Osteonecrosis lesions and triggering events.

Osteonecrosis type by medication: Patients N=191	MRONJ: n=98	<i>MRONJ cancer:</i> <i>(n=65)</i>	<i>MRONJ osteoporosis:</i> <i>(n=33)</i>	Non-MRONJ: n=93	<i>Non-MRONJ osteoradio-necrosis:</i> <i>(n=21)</i>	<i>Non-MRONJ Other osteonecrosis:</i> <i>(n=72)</i>
Anatomical region of the lesion: (n)						
Maxilla	10	8	2	0	0	0
Mandible	99	66	33	94	22	72
Additional separate lesions^{a)}	11	9	2	1	1	0
Triggering events^{b)}: (n)						
Tooth extraction	71	44	27	62	13	49 ^{c)}
Trauma/abrasive denture	11	9	2	6	1	5
Jaw surgery	5	2	3	8	5	3
Dental procedure	1	1	0	5	1	4
Jaw pathology	1	0	1	6	1	5
No triggering events^{d)}	20	18	2	7	1	6
- triggering event before antiresorptive medic.	22	11	11			
- triggering event during antiresorptive medic.	55	36	19			
		<i>mean ± SD</i>	<i>mean ± SD</i>		<i>mean ±SD</i>	<i>p-value</i>
Time (months) from triggering to dg/sampling		20±21	47±49		19±18	<i>NS</i>

Osteonecrosis type by medication: MRONJ: subgroups cancer (with bone metastases) and osteoporosis; non-MRONJ: subgroups osteoradionecrosis, other (causes of) osteonecrosis. a) The additional separate lesions are from different jaw areas and occurred simultaneously or at different times. b) cases of triggering events in the anatomical area of necrosis. c) infected tooth extractions in surgery, and biopsy for osteonecrosis diagnosis taken simultaneously – no other previous triggering events except infection and poor oral hygiene. d) number of patients. Statistics: *p-value* is measured with *Pearson Chi-square (2-sided sig)* between subgroups: cancer, osteoporosis, and osteoradionecrosis. Abbreviations: MRONJ, medication-related osteonecrosis of the jaw; *NS*, not significant.

Supplementary Table 3. Laboratory results (mean±SD) obtained before diagnostic debridement surgery.

Osteonecrosis type:	MRONJ n=109	Non-MRONJ n=94	<i>p=</i> value
<i>CRP (mg/l)</i>	19±30	32±66	<i>NS</i>
<i>leucocyte count (E9/l)</i>	12.1±21.3	10.5±16.7	<i>NS</i>
<i>Hb (g/l)</i>	123±16	136±17	<i><0.001</i>

Osteonecrosis type: MRONJ and non-MRONJ. Statistics: *p-value* is measured with *Pearson Chi-square (2-sided sig)*.

Abbreviations: MRONJ, medication-related osteonecrosis of the jaw; CRP, C-reactive protein; Hb, haemoglobin.

Supplementary Table 4. Results of the multivariable binary logistic regression model predicting the likelihood of Actinomyces infection (A—B), High MMP-8 levels (C—D), and acute inflammation.

A) Actinomyces infection, sample-level						A) Actinomyces infection, patient-level					
Variable	Coefficient	SE	OR	95% CI	p-value	Variable	Coefficient	SE	OR	95% CI	p-value
Sex, male (ref. female)	0.301	0.372	1.351	0.651–2.804	0.419	Sex, male (ref. female)	0.309	0.377	1.363	0.651–2.851	0.411
Age, year	0.019	0.013	1.019	0.993–1.045	0.147	Age, year	0.018	0.013	1.018	0.992–1.044	0.180
No. of comorbidities, 3 or more (ref. 2 or less)	-0.529	0.522	0.589	0.212–1.638	0.310	No. of comorbidities, 3 or more (ref. 2 or less)	-0.578	0.531	0.561	0.198–1.588	0.276
Current smoking, yes (ref. no)	0.340	0.405	1.405	0.636–3.108	0.400	Current smoking, yes (ref. no)	0.407	0.413	1.503	0.669–3.374	0.324
Steroid medication, yes (ref. no)	0.440	0.431	1.553	0.667–3.615	0.307	Steroid medication, yes (ref. no)	0.379	0.440	1.461	0.617–3.461	0.389
MRONJ group, yes (ref. non-MRONJ)	1.244	0.435	3.469	1.478–8.141	0.004	MRONJ group, yes (ref. non-MRONJ)	1.285	0.451	3.613	1.494–8.741	0.004
C) High MMP-8 levels, sample-level						C) High MMP-8 levels, patient-level					
Variable	Coefficient	SE	OR	95% CI	p-value	Variable	Coefficient	SE	OR	95% CI	p-value
Sex, male (ref. female)	-0.074	0.325	0.928	0.491–1.754	0.819	Sex, male (ref. female)	-0.207	0.334	0.813	0.423–1.563	0.534
Age, year	0.010	0.013	1.010	0.986–1.036	0.419	Age, year	0.009	0.013	1.010	0.984–1.035	0.465
No. of comorbidities, 3 or more (ref. 2 or less)	0.317	0.472	1.373	0.544–3.464	0.502	No. of comorbidities, 3 or more (ref. 2 or less)	0.422	0.494	1.525	0.579–4.020	0.393
Current smoking, yes (ref. no)	-0.437	0.361	0.646	0.318–1.310	0.226	Current smoking, yes (ref. no)	-0.484	0.371	0.617	0.298–1.275	0.192
Steroid medication, yes (ref. no)	-0.280	0.360	0.756	0.373–1.532	0.438	Steroid medication, yes (ref. no)	-0.222	0.374	0.801	0.385–1.668	0.553
MRONJ group, yes (ref. non-MRONJ)	0.749	0.380	2.115	1.004–4.458	0.049	MRONJ group, yes (ref. non-MRONJ)	0.716	0.394	2.047	0.945–4.432	0.069
E) Acute inflammation, sample-level						E) Acute inflammation, patient-level					
Variable	Coefficient	SE	OR	95% CI	p-value	Variable	Coefficient	SE	OR	95% CI	p-value
Sex, male (ref. female)	0.186	0.327	1.204	0.635–2.284	0.569	Sex, male (ref. female)	0.145	0.332	1.157	0.604–2.215	0.661
Age, year	0.025	0.013	1.025	1.000–1.051	0.048	Age, year	0.021	0.013	1.021	0.996–1.047	0.096
No. of comorbidities, 3 or more (ref. 2 or less)	0.173	0.457	1.188	0.486–2.908	0.705	No. of comorbidities, 3 or more (ref. 2 or less)	0.094	0.469	1.099	0.439–2.754	0.840
Current smoking, yes (ref. no)	0.833	0.373	2.300	1.107–4.777	0.026	Current smoking, yes (ref. no)	0.840	0.378	2.317	1.103–4.864	0.026
Steroid medication, yes (ref. no)	-0.036	0.355	0.965	0.481–1.936	0.920	Steroid medication, yes (ref. no)	-0.101	0.366	0.904	0.441–1.854	0.783
MRONJ group, yes (ref. non-MRONJ)	0.537	0.381	1.711	0.811–3.612	0.159	MRONJ group, yes (ref. non-MRONJ)	0.619	0.393	1.857	0.860–4.013	0.115
SE = standard error											
OR = odds ratio											
CI = confidence interval											