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CLINICAL RESEARCH

Post-treatment Apical Periodontitis: Histobacteriologic and Histopathologic Findings



SIGNIFICANCE

Histobacteriologic and histopathologic findings confirm that persistent intraradicular infection, often as biofilm, is the primary cause of posttreatment apical periodontitis, reinforcing the importance of complete disinfection and sealing of the apical root canal system in endodontic therapy.

ABSTRACT

Introduction: Occurrence of post-treatment apical periodontitis characterizes the failure of endodontic treatment. This study described the histobacteriologic and histopathologic features of biopsy specimens of teeth with post-treatment apical periodontitis. **Methods:** The material comprised sequential biopsy specimens of 71 human root canal-treated teeth with apical periodontitis. Specimens were obtained by apical surgery or extraction and consisted of root apices with attached lesions, which were processed for histobacteriologic and histopathologic evaluation of the cause of failure, infection distribution, and the type of lesion. Different aspects of infection were evaluated for associations with demographic, clinical, radiographic and histopathologic parameters. **Results:** Intraradicular bacteria were found in the apical root canal system of 94% of the specimens, often organized in biofilm structures (79% of the cases). Infection extended to dentinal tubules and ramifications in many cases. The occurrence of biofilms in the main apical canal lumen was significantly more frequent in teeth with underfillings and inadequate root canal treatment ($P < .05$). An extraradicular infection occurred in 37% of the cases; in all but one case, it was associated with an intraradicular infection. Extraradicular bacteria were observed as a biofilm (sometimes with calcification foci) attached to the outer root surface and as planktonic cells or flocs at the lesion body or in the cyst lumen. Extraradicular infection was significantly more present in cases with symptoms and sinus tracts, abscesses, large lesions, and retreatment ($P < .05$). Nonepithelialized granuloma was the most frequent type of post-treatment apical periodontitis (42%). **Conclusions:** Intraradicular infection was the major factor associated with post-treatment apical periodontitis. Extraradicular infection was observed in about one-third of the cases, and in the large majority of them associated with concomitant intraradicular infection. Granuloma was the most common histopathologic diagnosis of posttreatment lesions. (*J Endod* 2026;52:940–952.)

KEY WORDS

Apical cyst; biofilm; endodontic infection; extraradicular infection; post-treatment apical periodontitis

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Failure of the root canal treatment or retreatment is characterized by the persistence, emergence or recurrence of apical periodontitis after a given period of follow-up evaluation. Post-treatment apical periodontitis is the disease of root canal-treated teeth and is most often associated with treatment of substandard quality¹⁻³. In such cases, it is not difficult to realize that the cause of post-treatment disease is inadequate infection control during nonsurgical root canal treatment. Residual infection is a well-established risk factor for treatment failure^{4,5}, and it has been demonstrated that the bacterial counts in the apical canal of teeth with post-treatment disease are directly proportional to the volume of untreated canal^{6,7}.

Apical periodontitis can also fail to completely heal in some cases treated following acceptable standards. Post-treatment disease has been reported to occur in 1 out of 5–7 teeth that were treated/retreated because of a preoperative apical periodontitis lesion⁸⁻¹¹. Studies using culture or molecular microbiology techniques have revealed that bacteria are found in the canals of virtually all cases of posttreatment apical periodontitis¹²⁻¹⁵. Morphologic investigations¹⁶⁻²² have reported on the

TABLE 1 - Demographic and Clinical Characteristics of all Cases Included in the Study, with Indication of their Publication Status

Case No	Patient ID	Sex	Age (y)	Tooth/Root	Treatment type	Specimen source	Publication status	Reference no
1	VM	F	20	18/M	Primary	Extraction	Published	16
2	BG	F	38	13	Primary	Apical surgery	Published	16
3	MG	M	40	19/M	Primary	Extraction	Published	16
4	IF	F	30	6	Retreatment	Apical surgery	Published	16
5	AC	F	24	10	Primary	Extraction	Published	16
6	GM	F	39	6	Primary	Extraction	Published	16
7	ZS	F	23	9	Primary	Apical surgery	Published	16
8	SE	F	28	19/M	Primary	Apical surgery	Published	16
9	AD	F	38	10	Primary	Apical surgery	Published	16
10	IA	F	20	14/D	Primary	Extraction	Published	16
11	SML	F	25	10	Primary	Extraction	Published	16
12	GL	M	14	10	Primary	Apical surgery	Published	16
13	CG	F	25	10	Primary	Apical surgery	Published	16
14	SG	M	45	19/M	Primary	Extraction	Published	16
15	DG	M	39	12	Primary	Extraction	Published	16
16	PM	M	45	8	Retreatment	Apical surgery	Published	16
17	KN	F	21	28	Primary	Apical surgery	Published	16
18	LP	F	51	5	Retreatment	Apical surgery	Published	16
19	AC	F	32	7	Primary	Apical surgery	Published	16,30
20	GS	F	23	14/M	Retreatment	Apical surgery	Published	16
21	IS	F	28	24	Retreatment	Apical surgery	Published	16
22	LS	M	31	4	Retreatment	Apical surgery	Published	16
23	RE	F	24	14/M	Primary	Apical surgery	Published	16
24	AR	F	23	13	Primary	Extraction	Published	16
25	GE	M	48	9	Primary	Apical surgery	Unpublished	—
26	LM	F	22	8	Primary	Apical surgery	Unpublished	—
27	SS	F	19	30/M	Primary	Extraction	Unpublished	—
28	PS	F	47	12	Primary	Apical surgery	Unpublished	—
29	PA	M	41	28	Primary	Apical surgery	Unpublished	—
30	BB	M	44	31	Retreatment	Extraction	Unpublished	—
31	LM	M	20	30/M	Primary	Apical surgery	Unpublished	—
32	ST	M	22	9	Primary	Apical surgery	Unpublished	—
33	CC	F	37	19/M	Retreatment	Apical surgery	Unpublished	—
34	TV	F	38	4	Primary	Extraction	Unpublished	—
35	MAF	F	39	30/M	Primary	Extraction	Unpublished	—
36	RE	M	67	18/M	Primary	Extraction	Unpublished	—
37	VA	F	40	10	Primary	Apical surgery	Unpublished	—
38	CM	M	37	7	Primary	Apical surgery	Published	20
39	SA	M	35	24	Retreatment	Apical surgery	Published	23
40	LW	M	42	25	Retreatment	Apical surgery	Published	23
41	SA	M	30	30/D	Primary	Extraction	Unpublished	—
42	LWJ	M	42	9	Primary	Apical surgery	Published	23
43	LL	M	63	8	Primary	Extraction	Unpublished	—
44	AM	M	51	14/M	Retreatment	Apical surgery	Published	21
45	MS	F	45	31/D	Primary	Extraction	Unpublished	—
46	CLP	F	53	4	Primary	Extraction	Unpublished	—
47	BC	M	70	29	Primary	Extraction	Unpublished	—
48	CG	M	29	7	Primary	Apical surgery	Unpublished	—
49	MC	F	50	8	Primary	Apical surgery	Unpublished	—
50	AG	M	18	19/D	Primary	Extraction	Unpublished	—
51	BC	M	15	24	Primary	Apical surgery	Unpublished	—
52	KD	M	44	14/M	Primary	Apical surgery	Unpublished	—
53	WL	F	17	9	Primary	Apical surgery	Published	29
54	CD	M	41	14/M	Primary	Apical surgery	Unpublished	—
55	RE	M	63	10	Primary	Extraction	Unpublished	—
56	CL	M	34	11	Primary	Apical surgery	Unpublished	—
57	VS	M	55	4	Primary	Apical surgery	Unpublished	—
58	LS	F	77	21	Primary	Apical surgery	Published	31
59	LJ	M	43	30/M	Retreatment	Apical surgery	Unpublished	—
60	RA	F	51	6	Retreatment	Apical surgery	Unpublished	—
61	OC	M	20	5/B	Primary	Extraction	Unpublished	—
62	LU	M	38	8	Primary	Extraction	Unpublished	—

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TABLE 1 - Continued

Case No	Patient ID	Sex	Age (y)	Tooth/Root	Treatment type	Specimen source	Publication status	Reference no
63	IR	F	36	20	Primary	Extraction	Unpublished	—
64	TM	F	58	6	Primary	Extraction	Unpublished	—
65	PG	F	30	10	Retreatment	Apical surgery	Unpublished	—
66	EA	M	21	5	Primary	Extraction	Unpublished	—
67	GV	M	28	4	Primary	Extraction	Unpublished	—
68	MC	F	45	10	Retreatment	Apical surgery	Unpublished	—
69	MV	M	31	14/DB	Primary	Extraction	Unpublished	—
70	GD	F	45	7	Primary	Apical surgery	Unpublished	—
71	CA	M	53	19	Primary	Extraction	Unpublished	—

occurrence of residual bacterial infection in the apical segment of the root canal system of teeth with posttreatment disease, often organized in intraradicular biofilm structures^{16,18}. There are also reports of extraradicular infection as an occasional cause of persistent apical periodontitis²³⁻²⁶. Finally, because some studies found no bacteria in association with a few cases of post-treatment disease, a theory was raised that nonmicrobial factors, including foreign-body reaction and true cysts, could be the cause of persistent inflammation^{27,28}.

Previous evaluations of causes of failure of nonsurgical root canal treatment/retreatment were usually based on case series^{16,27} or used analytical techniques with significant limitations, including the failure to perform serial sections of the biopsy specimens and the failure to preserve the original spatial relationship between the root apex and the lesion during sample taking. To overcome these limitations, the present study was intended to report on the histobacteriologic and histopathologic analyses of a large number of biopsy specimens of teeth with posttreatment apical periodontitis, for which serial sections of root apexes with the attached lesions were available.

MATERIAL AND METHODS

Clinical Specimens

The study material comprised sequential biopsy specimens of 71 human root canal-treated teeth with apical periodontitis lesions. Biopsies originated from 71 patients (36 females and 35 males), aged 14–70 years (mean, 37 years), with a non-contributory medical history. The evaluated specimens consisted of the roots or root tips with attached apical periodontitis lesions from root canal-treated teeth that were subjected to

either root-end resection during periradicular surgery ($n = 42$) or tooth extraction ($n = 29$). Some of the included cases were previously published as case reports or series^{16,20,21,23,29-31} (Table 1). Informed consent was obtained from all patients.

Apical surgery was indicated because the previous apical periodontitis lesion had not responded favorably to nonsurgical root canal treatment. Extraction was indicated for root canal-treated teeth with apical periodontitis that were considered as nonrestorable or because the patient did not agree with further attempts to save the tooth. For the specimens obtained by apical surgery, a full mucoperiosteal flap was raised and the cortical bone was removed so as to allow access to the root apex and the apical periodontitis lesion. The root apex was cut and care was taken to remove the root tip with the inflammatory lesion attached. Actually, for inclusion of the specimens in the study, the apical periodontitis lesion had to remain attached to the root apex after extraction or apical surgery, with their original spatial relationship maintained.

All cases of post-treatment apical periodontitis were regarded as treatment/retreatment failure because a preoperative lesion persisted and remained unaltered or increased in size with at least 1-year follow-up; signs and symptoms of infection developed; or a new lesion emerged. Most biopsy specimens evaluated in this study (48) were obtained from consecutive cases evaluated and treated by one of the authors (D.R.) in a private endodontic practice. Twenty-three cases were treated by 16 experienced endodontists who sent the specimens for histobacteriologic evaluation.

The following clinical and radiographic data were recorded: presence and intensity (mild, moderate or severe) of symptoms, radiographic lesion size, sinus tract, type of

intervention (treatment/retreatment), apical limit and quality of root canal filling, and presence and quality of coronal restoration. Lesion size was measured as the largest diameter of the apical periodontitis lesion on periapical radiographs and was classified as small (<5 mm), large (≥ 5 mm and <10 mm) or very large (≥ 10 mm). Teeth with an endodontic-periodontal lesion or root fracture were excluded.

Histobacteriologic and Histopathologic Analyses

The biopsy specimens were immediately placed in 10% buffered formalin for fixation for at least 48 hours. Next, the specimens were demineralized in 22.5% (vol/vol) formic acid and 10% (wt/vol) sodium citrate for 3–4 weeks. The demineralization end-point was determined radiographically. The demineralized specimens were washed in running water for 24 hours, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin. Care was taken for the specimens to be cut in such a way to include the apical root canal and the apical periodontitis lesion in direct continuity, preserving their spatial relationship. Serial sections were taken and every fifth slide was stained with hematoxylin and eosin for histopathologic evaluation. The other sections were subjected to histobacteriologic analyses by using the Taylor modified Brown and Brenn staining method³².

The histopathologic and histobacteriologic aspects were evaluated as follows:

1. Intraradicular infection: bacteria present in the apical segment of the root canal system, including the main canal lumen, dentinal walls and apical ramifications; bacterial occurrence as planktonic cells or structured in biofilms (bacterial populations

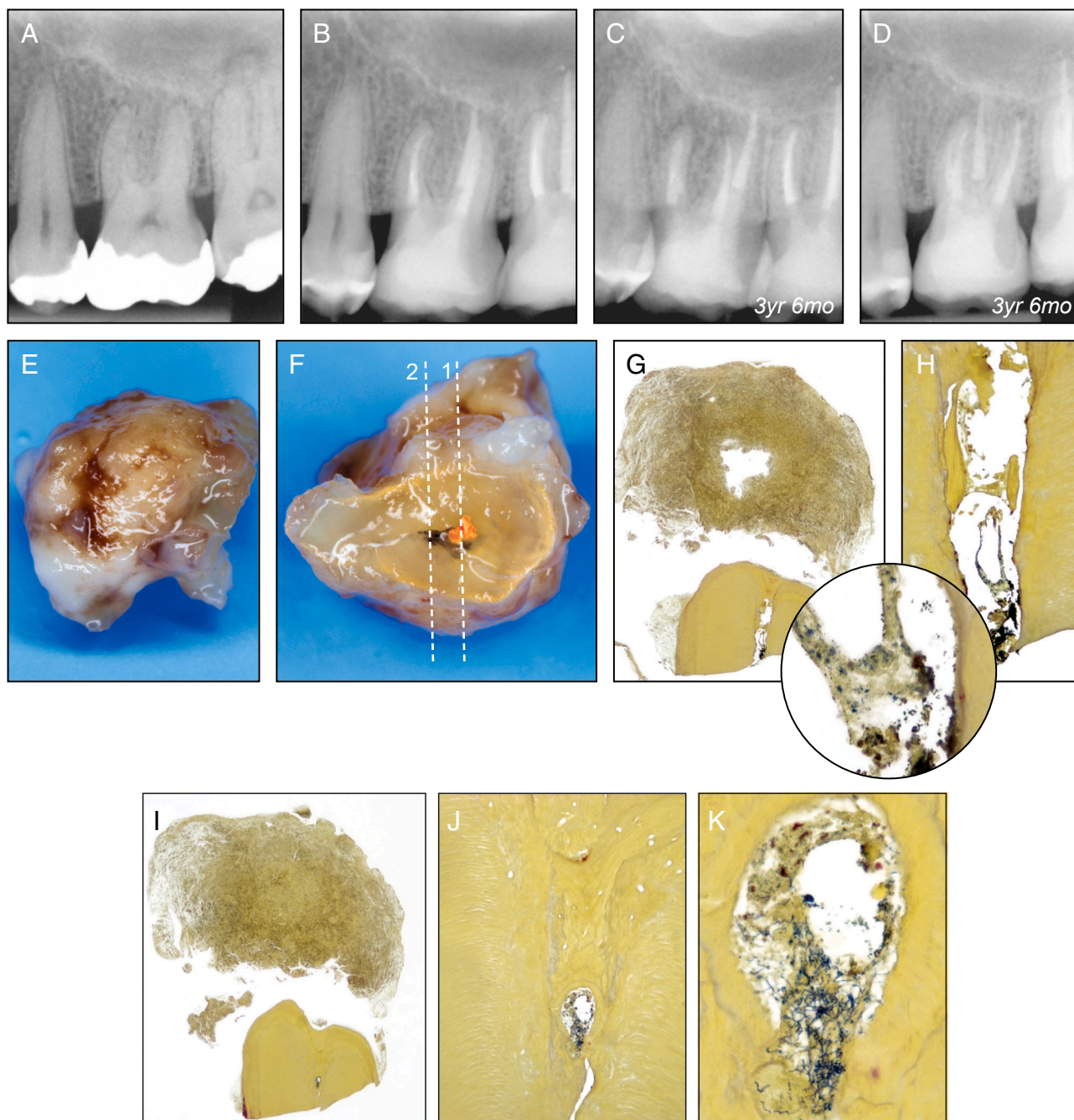


FIGURE 1 – (Case # 54, [Table 1](#)). Forty-one-year-old man referred for endodontic treatment of maxillary left first and second molar for pulp necrosis caused by recurrent caries around golden overlays. The patient declared intermittent spontaneous pain in the second quadrant. (A) Diagnostic radiograph showing early periapical disturbances. Based on clinical examination, the diagnosis of pulp necrosis was made for both teeth. After removal of restorative materials and decay, pulp exposure was observed, with partly liquefied bleeding tissue in the chamber of tooth #14. Root canal treatment was completed in 3 sessions with calcium hydroxide as an interim intracanal medicament. The second mesiobuccal canal (MB2) was found to have an independent course and foramen. Canals were filled with lateral compaction and an epoxy resin-based sealer. Immediate adhesive build-up followed. (B) Postoperative radiograph. The tooth was finally restored with a ceramic overlay. (C and D) The patient returned after 3 years and 6 months declaring that in the last 6 months the tooth was uncomfortable. At examination the tooth was tender to vertical and lateral percussion. Periapical radiographs taken with different angulation showed that the periapical radiolucency on MB root had increased considerably. Root-end resection was decided. (E and F) The root tip and the pathologic periapical tissue were retrieved in their original relationships. The specimen was immersed in formalin for histologic analysis. (G) Longitudinal section cut through MB1, at the level of line 1 in (F), encompassing the apical canal and the periapical pathologic tissue, showing that the lesion had an apparently empty cavity centrally, surrounded by a concentration of PMNs, owing to the histologic diagnosis of apical abscess (Taylor-modified Brown and Brenn, orig. mag. $\times 16$). (H) Detail of the apical canal, containing necrotic debris colonized by bacteria (orig. mag. $\times 100$; inset $\times 400$). (I) Section cut at the level of the line 2 in (F) showing a partly calcified apical canal (orig. mag. $\times 16$). (J and K) Progressive magnification of the apical canal. A space is present, filled with debris and severe bacterial colonization (orig. mag. $\times 100$ and $\times 400$). (Clinical management by Dr. Daniel Cerny).

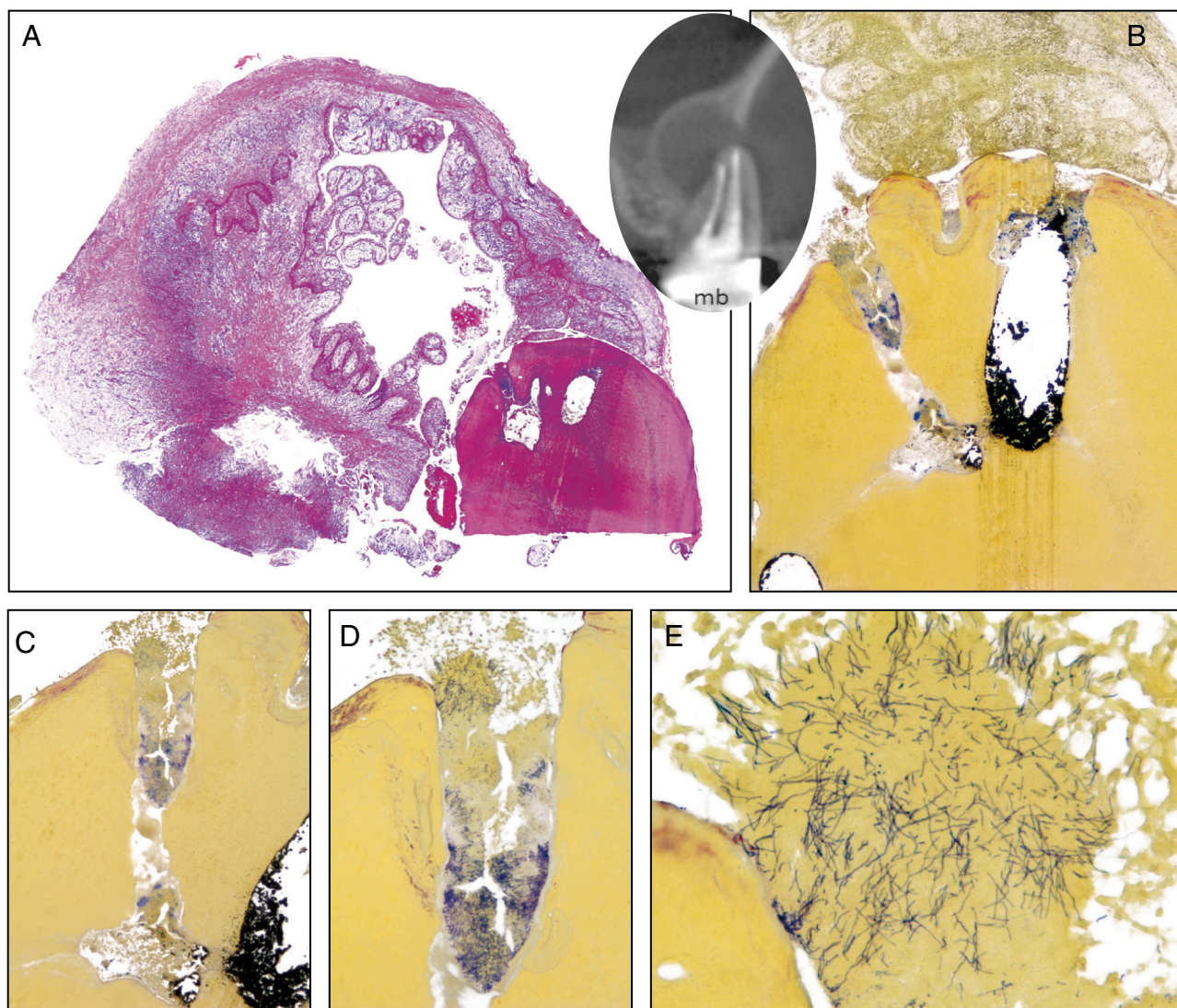


FIGURE 2 – (Case # 44, [Table 1](#)). Maxillary first molar from a 51-year-old male patient with necrotic pulp and apical periodontitis lesions affecting all roots. The tooth was initially treated by an endodontic specialist; however, the lesion on the mesiobuccal (MB) root increased in size after 18 months (inset), accompanied by clinical symptoms. Apical microsurgery was therefore performed. The apex of the MB root was surgically removed in one block along with the large periapical lesion. (A) Histologic section of the MB root apex cut in a bucco-lingual plane. Overview showing a large cystic cavity continuous with an apical canal ramification. The lesion was classified as a “bay cyst” (H&E, orig. mag. $\times 2$). (B) Section obtained 40 levels deeper than (A), stained with the Taylor-modified Brown and Brenn method. The lumen of the MB1 canal and its ramifications appear clogged with bacterial biofilms (orig. mag. $\times 16$). (C–E) Progressive magnifications of the course and exit of the left ramification shown in (B). The lumen is filled with thick bacterial biofilms. Intertwining, ramifying bacteria dominate the foraminal opening, extending into the cyst lumen (orig. mag. $\times 50$, $\times 100$, and $\times 400$). (Modified from Arnold et al²¹, with permission).

- attached to a surface and enmeshed in an extracellular polymeric substance matrix).
2. Extraradicular infection: bacteria present within the body of the apical periodontitis lesion (as planktonic cells or large colonies/flocs), within the lumen of the cyst cavity (as planktonic cells or large colonies/flocs), or attached to the outer apical root surface (biofilm).
3. Inflammation and epithelial proliferation; presence and distribution of acute and chronic inflammatory cells, and epithelial cells. Lesions were histopathologically diagnosed as abscess, granuloma, or

cyst. Abscesses and granulomas were subclassified as epithelialized or nonepithelialized. Apical cysts were subclassified as true (isolated cystic cavity not continuous with the root canal) or bay (cystic cavity in a continuum with the root canal).

Statistical Analysis

Prevalence of intraradicular and extraradicular infection in teeth with posttreatment apical periodontitis was evaluated in general and according to clinical features and

histopathologic diagnosis. Different aspects of intraradicular infections, including presence of bacteria as planktonic cells or biofilm structures in the main apical canal and biofilms in ramifications, were evaluated for associations with the following parameters: sex, age, previous intervention type (treatment/retreatment), intensity of symptoms, sinus tract, lesion size, apical limit and quality of filling, presence and quality of the coronal restoration, and histopathologic diagnosis. Aspects related to extraradicular infection, such as the occurrence of a bacterial biofilm/calculus on the outer root

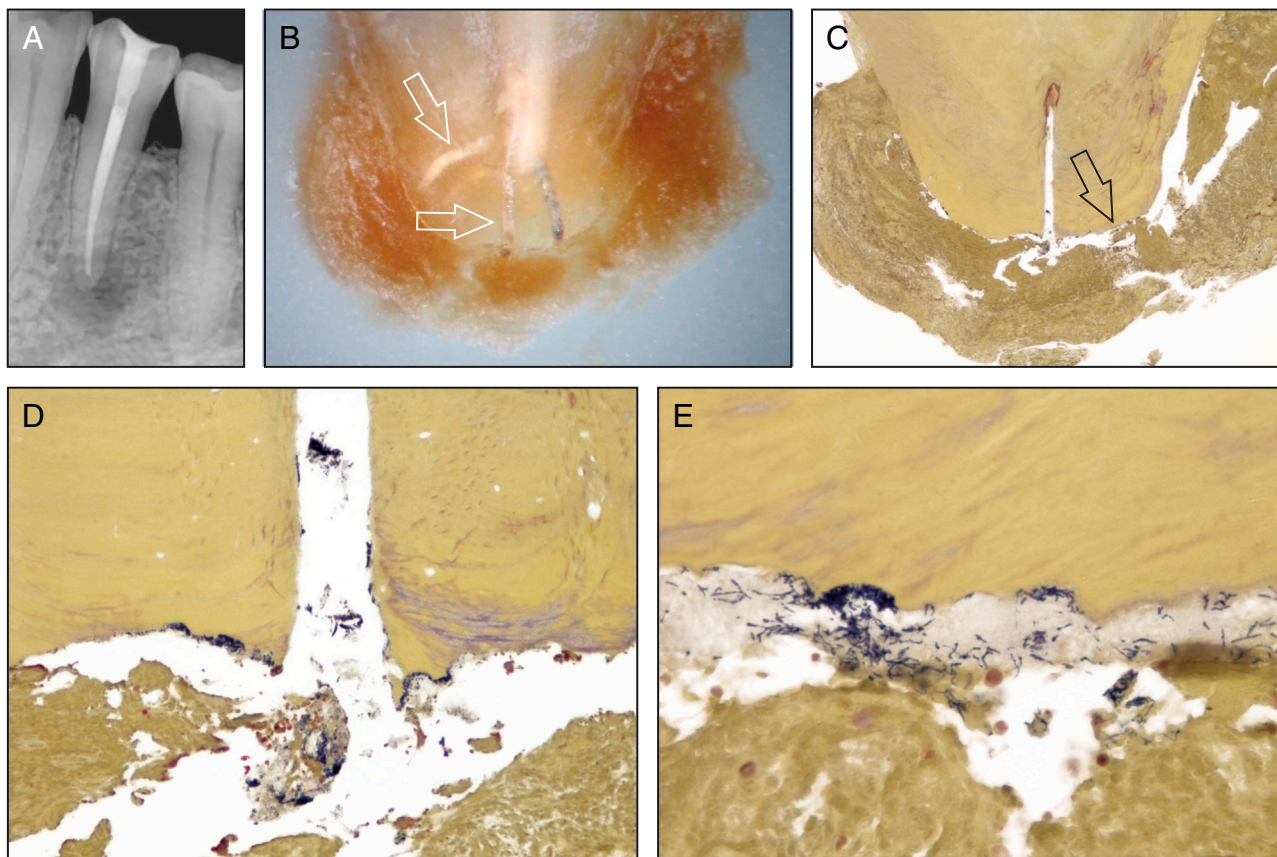


FIGURE 3 – (Case # 58, Table 1). (A) Mandibular first premolar in a 77-year-old woman, with necrotic pulp and a buccal sinus tract. After 3 sessions of canal instrumentation including ultrasonic activation of 5% sodium hypochlorite and 17% EDTA, and calcium hydroxide medication, the sinus tract remained unchanged. In the presence of a condition refractory to the orthograde procedures for infection control, an intervention of apical microsurgery was deemed necessary. Preliminarily, the canal was filled with cold lateral compaction of gutta-percha and a bioceramic sealer and a radiograph was taken. This revealed the separated tip of a K-file apical to the canal filling. (B) Photograph of the root tip in the paraffin block. Several apical ramifications can be seen. The separated tip of a small file is in one ramification. (C) Section cut through the ramification ending at the center of the apex (*lower arrow* in [B]). Overview (Taylor-modified Brown and Brenn, orig. mag. $\times 16$). (D) Intermediate magnification of the foraminal opening in (C). Bacterial biofilms are present in the canal lumen, in the periapical tissue, and on the external apical surface (orig. mag. $\times 100$). (E) High power view of the area of the apical surface indicated by the arrow in (C). A biofilm is present, filling the irregularities of the resorbed apical surface. Note that the extracellular polymeric component prevails over the bacterial one (orig. mag. $\times 400$). (F) Section cut approximately 80 sections away from that in (C), encompassing 2 ramifications (orig. mag. $\times 16$). (G) Intermediate magnification of the area marked in (F). Lumen is clogged with bacteria. Another cross cut ramification is present (orig. mag. $\times 100$). (H) High power view from the left portion of the ramification in (G) (orig. mag. $\times 400$). (I) High power view of the cross cut ramification in (G) (orig. mag. $\times 400$). (Modified from Ricucci et al³¹, with permission).

surface, bacteria in the cyst lumen and/or in the lesion body as planktonic cells or flocs, were also evaluated for associations with the same clinical, demographic, radiographic and histopathologic parameters. Data were reported in a descriptive way and dichotomized for some variables involving 3 or more conditions, as follows: asymptomatic (absent symptoms) versus symptomatic teeth (mild + moderate + severe); small versus large + very large lesions; adequate versus inadequate + absent coronal restoration; and abscess (epithelialized + nonepithelialized) versus granuloma (epithelialized + nonepithelialized) versus cyst (bay + true). Data were evaluated by the chi-square and the Fisher's exact tests. The level of significance was established at 5% ($P < .05$).

RESULTS

In general, histobacteriologic evaluation revealed intraradicular bacteria in the apical root canal system of 67 out of 71 biopsy specimens (94%) (Figs. 1–3). Intraradicular bacterial biofilms were visualized on the dentinal walls of the main apical canal lumen of 56 teeth (79%) (Figs. 1G–K, 2B, 3C and D). In 46 teeth (65%), biofilms were seen inside apical ramifications (Fig. 2B–D and 3F–I). Planktonic bacteria were visualized somewhere in the apical canal lumen in 60 teeth (84.5%). In many cases, bacteria at the bottom of biofilm structures were seen within dentinal tubules at varying depths.

Of the 4 cases that bacteria were not detected within the canal system, one presented with an extraradicular infection

containing actinomycotic colonies while the other 3 showed no bacteria in the extraradicular space either (Fig. 4). All these 4 cases were histopathologically diagnosed as bay cysts. Of the 3 cases in which bacteria were not found anywhere, 2 exhibited a severe concentration of polymorphonuclear leukocytes (PMNs) at the apical foramen (Fig. 4E and F). This strongly suggests that intraradicular bacteria were actually present; they were either lost during processing or located more coronally in the canal (Fig. 4E and F). The third case with no bacteria showed inflammation around a large amount of extruded filling material.

Evaluation of the association of the different patterns of intraradicular infection with demographic, clinical, radiographic, and histopathologic factors revealed that the

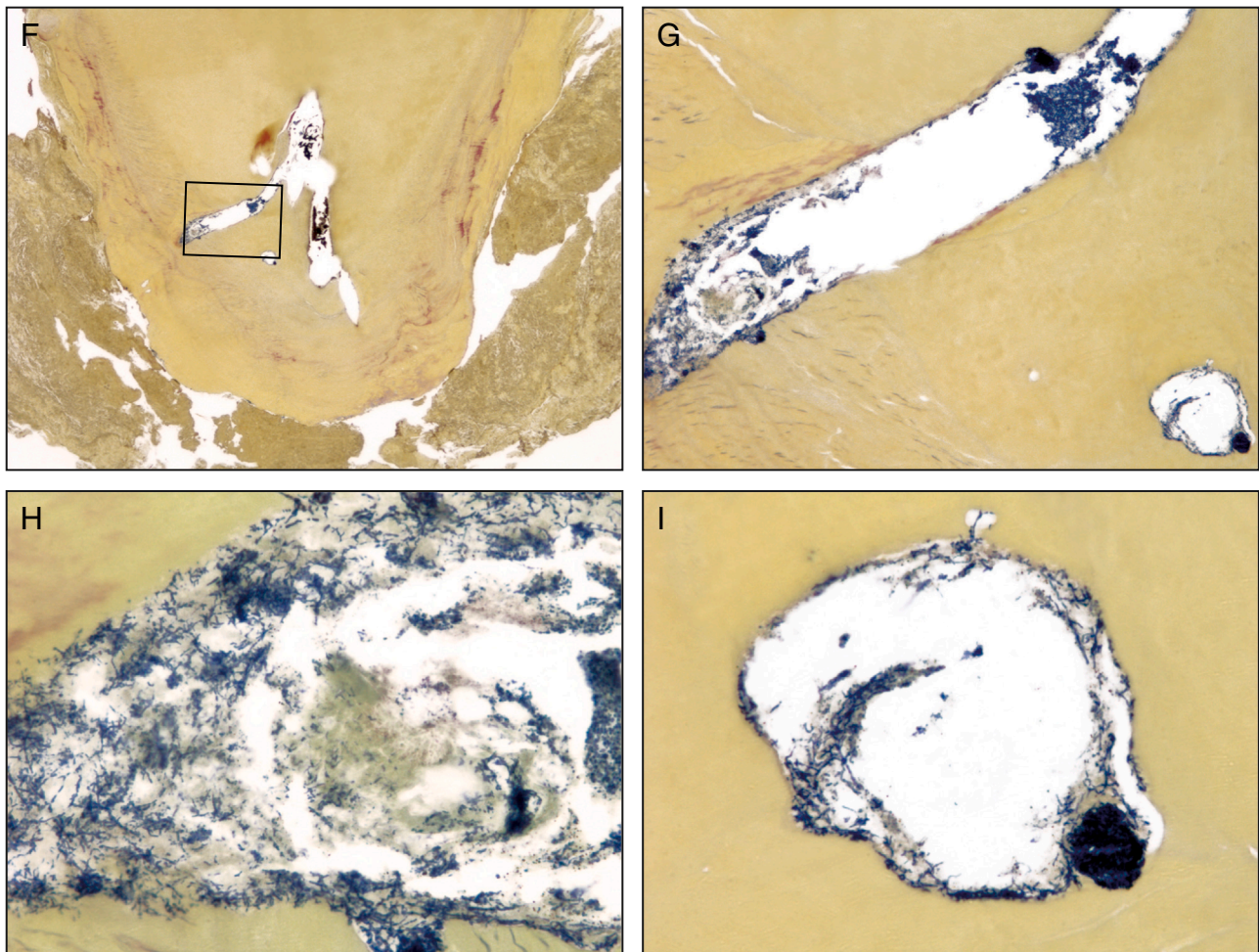


FIGURE 3 – Continued

occurrence of biofilms in the main apical canal lumen was significantly more frequent along with underfillings and inadequate root canal treatment ($P < .05$). The presence of biofilms within apical ramifications was significantly higher in male patients, symptomatic teeth, underfillings and inadequate root canal treatment ($P < .05$). Planktonic bacteria in the main apical canal lumen were significantly more frequent in abscesses and granulomas than cysts ($P < .05$).

Overall, an extraradicular infection occurred in 26 of 71 (37%) of the cases. In all but one case with an extraradicular infection, there were also intraradicular bacteria, either in the main canal lumen or in apical ramifications, or both (Fig. 3). In 23 (32%) teeth, a biofilm was seen attached to the outer root surface (Fig. 3C–E). Biofilms showed evidence of calcification in 6 of these cases, forming a calculus (26% of the 23 biofilm cases, 8.5% of the 71 cases examined). In 25 cases, planktonic bacterial cells or flocs were visualized at the body of the lesion near the

exit of the apical foramen and/or apical ramifications (Fig. 3D). Bacteria were detected in the lumen of 4/13 cysts (31%). The only one true cyst observed in this study showed infection in the apical canal in the form of planktonic cells and biofilm structures on the dentinal walls and inside apical ramifications, with no extraradicular bacteria.

An extraradicular biofilm was significantly more present in cases with symptoms and sinus tracts, abscesses, large lesions, and retreatment ($P < .05$). Planktonic cells or flocs in the lesion body were significantly associated with retreatment, symptomatic, and large lesion cases ($P < .05$).

Histopathologic diagnosis revealed that the most common posttreatment apical periodontitis diagnoses were in descending order: nonepithelialized granuloma (42%), nonepithelialized abscess (22.5%), bay cyst (17%), epithelialized granuloma (10%), epithelialized abscess (7%), and true cyst (1%).

Data are depicted in Tables 2 and 3.

DISCUSSION

This histobacteriologic and histopathologic study evaluated the causes of root canal treatment failure as determined by the occurrence of posttreatment apical periodontitis. Biopsy specimens of the apical root and its associated apical periodontitis lesion were examined for the presence and pattern of intraradicular and extraradicular infections, as well as for the histopathologic type of lesion. For a more reliable evaluation¹⁷, the specimens were obtained with the apical periodontitis lesion in the preserved spatial anatomic relationship with the root apex and were subjected to serial sectioning.

Intraradicular infection was the most common probable cause of posttreatment disease, being observed in 94% of the cases. This is in consonance with the findings of many other studies using histology and different technologies to evaluate failure cases^{16,33-37}. Intraradicular infection

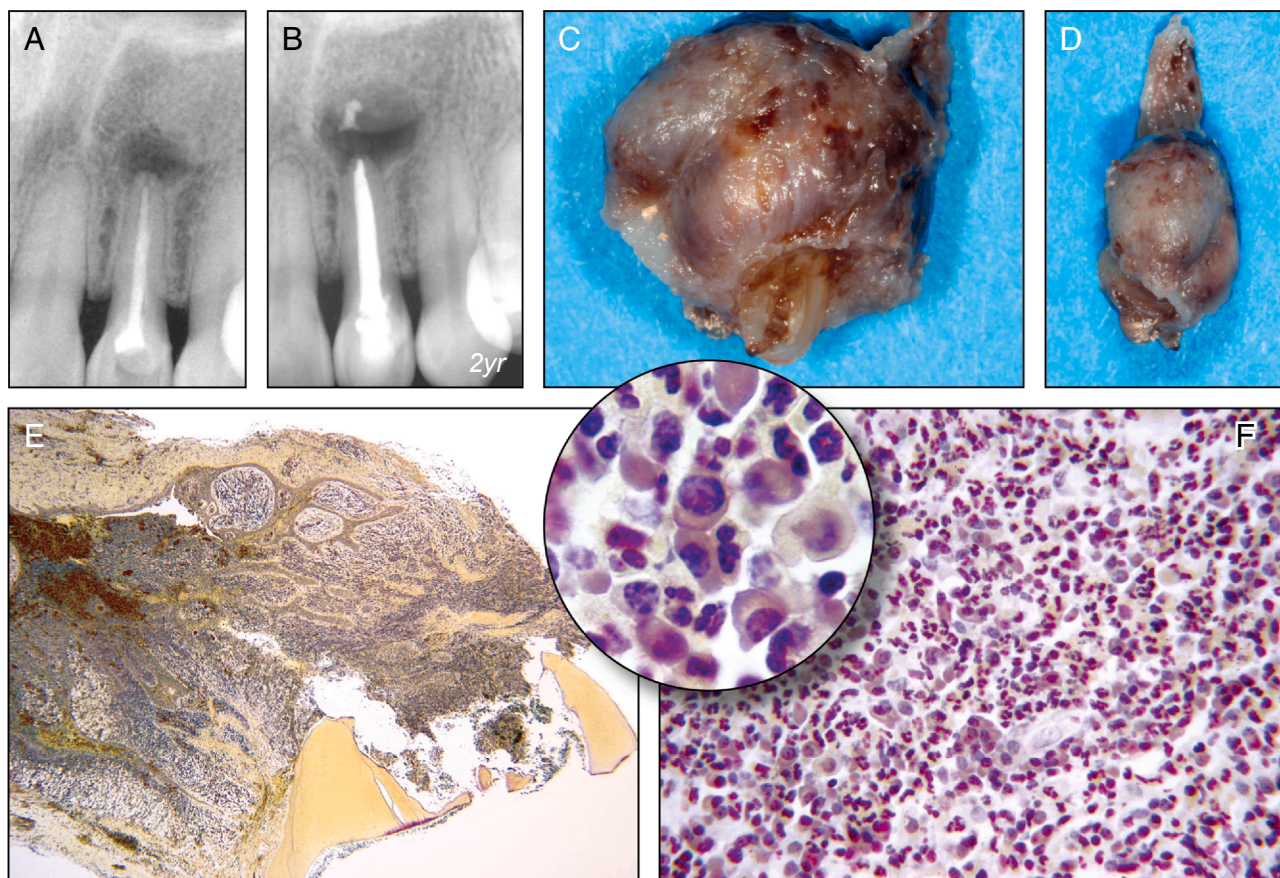


FIGURE 4— (Case # 68, Table 1). (A) Root canal–treated maxillary lateral incisor in a 45-year-old female. The tooth had been treated 10 years earlier, according to the patient, and had recently become symptomatic. The radiograph revealed a substandard root canal treatment and an apical periodontitis lesion. The tooth was retreated at the university endodontic department. (B) Two years later, the patient returned with pain during chewing. A radiograph showed considerable enlargement of the lesion, and apical microsurgery was planned. (C and D) Photographs of the biopsy specimen taken at different angles. The root tip and surrounding pathologic tissue were retrieved *en bloc*. (E) Section cut approximately through the center of the apical canal, including the foramen and adjacent periapical lesion (Taylor-modified Brown and Brenn, orig. mag. $\times 16$). (F) High-power view of the foramen area showing dense infiltration of acute and chronic inflammatory cells. No stainable bacteria were detected in any part of the apical canal or periapical tissue. This lesion was classified as a bay cyst (orig. mag. $\times 400$; inset $\times 1000$). (G) Section cut approximately 120 sections distant from (E), not encompassing the root tip. A cavity containing semi-solid material and lined by an epithelial layer is evident (orig. mag. $\times 16$). (H) High-power view of the epithelial lining. Stratified squamous epithelium infiltrated by acute and chronic inflammatory cells is observed. No bacteria are detectable (orig. mag. $\times 400$; inset $\times 1000$). (Clinical management by Dr. Elizeu A. Pascon).

associated with post-treatment disease can be caused by bacteria that endured the previous treatment (persistent infection) or that penetrated the canal during or after treatment, usually by a breach in the aseptic chain or coronal leakage (secondary infection)³⁸. Bacteria causing intraradicular infection occurred as planktonic cells floating in the lumen of the main canal and ramifications, as well as sessile communities (biofilms) attached to the dentinal walls. Biofilms were observed on the main apical canal lumen walls in 79% of the teeth, extending to ramifications in 65% of the cases. This confirms that biofilm organizations are commonly observed in the apical canal fragment of teeth with posttreatment apical periodontitis¹⁸. It is possible to infer that these were residual biofilms probably present on

root canal walls that may have been left unprepared after the previous treatment/retreatment³⁹. They may also have been resultant of microleakage through defective/absent coronal restorations⁴⁰.

Intraradicular biofilms located in the apical segment of the root canal system were positively associated with teeth showing underfillings, inadequate root canal treatment and symptoms. This is in line with previous studies using correlative cone-beam computed tomography, microcomputed tomography and molecular microbiology analyses that revealed the association of the volume of unfilled voids with higher bacterial counts, large lesions, and symptoms^{6,7}. Planktonic bacteria were found more frequently in the main apical canal segment in abscesses and granulomas

than cysts, and this may be related to the fact that these histopathologic conditions usually represent earlier stages of infection than cysts.

An extraradicular infection was associated with 37% of the cases of posttreatment apical periodontitis. Extraradicular bacteria occurred as planktonic or large colonies (flocs) in the lesion body or within the cyst lumen, or as a biofilm attached to the outer root surface. In 26% of the cases with an extraradicular biofilm, foci of calcification were found to give rise to an apical calculus. Extraradicular infections were virtually always (except for one case) associated with intraradicular infection. Therefore, it seems that only one case of independent extraradicular infection was observed in this

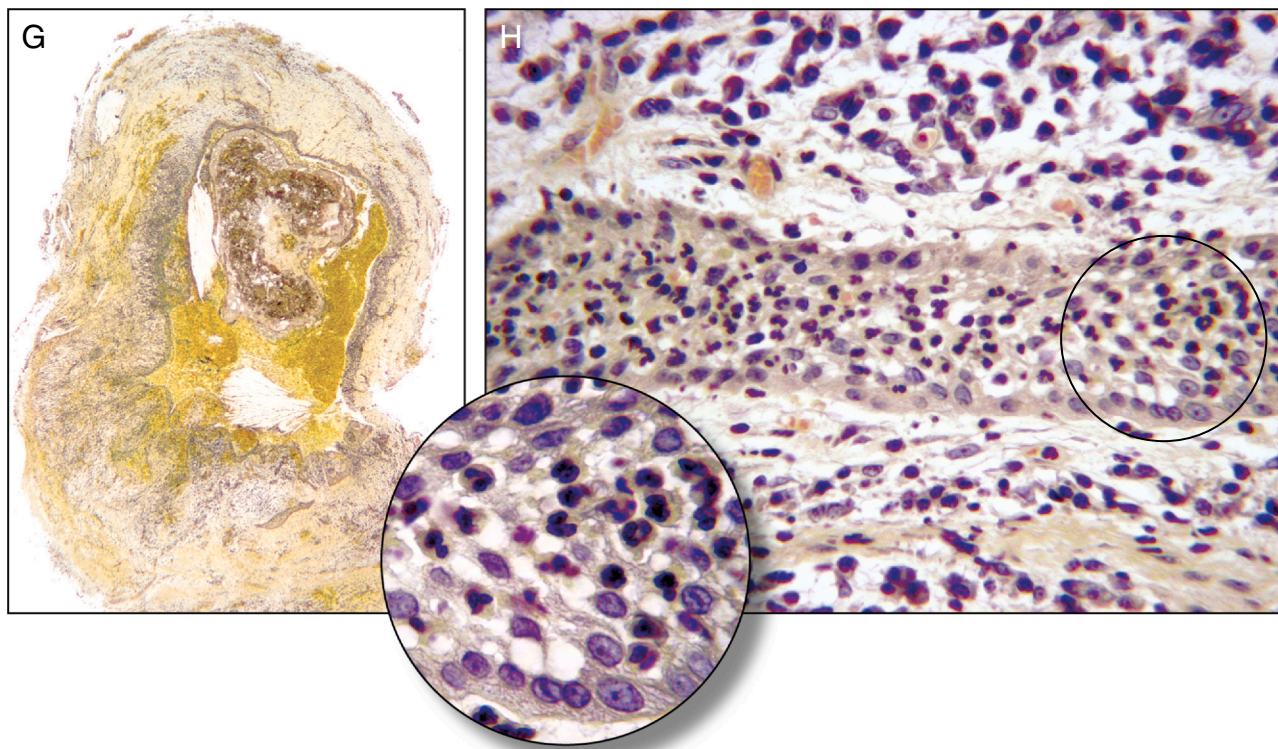


FIGURE 4 – Continued

study, which is in agreement with the concept that this condition is not very usual and can only be resolved by surgery²⁹.

Extraradicular biofilms were more often observed in teeth with symptoms, sinus tracts, abscesses, large lesions, and previous retreatment. Planktonic cells or flocs in the lesion body were more frequently found in teeth with symptoms, large lesions and previous retreatment.

For a more accurate histopathologic diagnosis, serial sectioning of the biopsy specimens should be performed, as was done in this study. Granuloma was the most common histopathologic diagnosis among the post-treatment apical periodontitis biopsies. In most specimens diagnosed as granuloma, there was no evidence of epithelial proliferation. This agrees with other studies evaluating primary and posttreatment disease and confirms that granuloma is the most common type of apical periodontitis^{41,42}. Cysts corresponded to 18% of the posttreatment lesions. The only one true cyst found in this study was associated with heavy infection in the apical canal system. Radiographically, all cysts were large/very large, that is, they

had more than 5 mm in diameter. Curiously, 22 of the 37 (59%) lesions classified as granulomas were large and 4 (11%) were very large. These findings indicate that cysts are virtually always large lesions, but not all large lesions are cysts.

Only 4 cases showed no intraradicular bacteria, all of them diagnosed as bay cysts. Of these, one had an extraradicular infection by actinomycotic-like bacterial organizations, and 2 others exhibited high accumulations of PMNs around and at the apical foramen. Because PMN accumulation is commonly observed near areas of infection by extracellular bacteria, these findings suggest the presence of intraradicular bacteria that may have gone undetected by the histobacteriologic approach, either being lost during processing or located more coronally in the canal. The third case with no bacteria showed inflammation around an enormous overfilling, which may represent a foreign-body reaction to the filling material. Thus, only one case evaluated in this study might have shown a nonmicrobial cause for posttreatment apical periodontitis. However, the possibility also exists that

intra or extraradicular bacteria may have passed unnoticed by the methodology used, as they may have been in previous investigations that suggested a nonmicrobial cause for post-treatment apical periodontitis²⁷. In the absence of irrefutable evidence for nonmicrobial cause of failure and based on the findings of this study, the primary cause of post-treatment disease is bacterial infection in or outside the root canal system.

In conclusion, intraradicular infection was the major factor associated with post-treatment apical periodontitis. Extraradicular infection was observed in approximately one-third of the cases, and in the large majority of them associated, and probably maintained, by a concomitant intraradicular infection. Granuloma was the most common histopathologic diagnosis of posttreatment lesions, corresponding to approximately one-half of the cases.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Domenico Ricucci: Conceptualization, Methodology, Investigation. **Andrea F.**

TABLE 2 - Prevalence of Intraradicular Infection in Teeth with Post-treatment Apical Periodontitis According to Clinical Features and Histopathologic Diagnosis

Variables	n = 71	Planktonic bacteria in the main apical canal		Biofilms in the main apical canal		Biofilm in apical ramifications	
		Absent	Present	Absent	Present	Absent	Present
Total	71 (100%)	11 (15.5%)	60 (84.5%)	15 (21.1%)	56 (78.9%)	25 (35.2%)	46 (64.8%)
Specimen obtained by							
Extraction	29 (40.8%)	1 (3.4%)	28 (96.6%)	2 (6.9%)	27 (93.1%)	6 (20.7%)	23 (79.3%)
Apicoectomy	42 (59.2%)	10 (23.8%)	32 (76.2%)	13 (31%)	29 (69%)	19 (45.2%)	23 (54.8%)
Sex							
Male	35 (49.3%)	3 (8.6%)	32 (91.4%)	5 (14.3%)	30 (85.7%)	8 (22.9%)	27 (77.1%)
Female	36 (50.7%)	8 (22.2%)	28 (77.8%)	10 (27.8%)	26 (72.2%)	17 (47.2%)	19 (52.8%)
Age (y)							
≤25	20 (28.2%)	2 (10%)	18 (90%)	5 (25%)	15 (75%)	9 (45%)	11 (55%)
26–40	24 (33.8%)	5 (20.8%)	19 (79.2%)	4 (16.7%)	20 (83.3%)	8 (33.3%)	16 (66.7%)
≥40	27 (38.0%)	4 (14.8%)	23 (85.2%)	6 (22.2%)	21 (77.8%)	8 (29.6%)	19 (70.4%)
Intervention							
Treatment	56 (78.9%)	6 (10.7%)	50 (89.3%)	11 (19.6%)	45 (80.4%)	19 (33.9%)	37 (66.1%)
Retreatment	15 (21.1%)	5 (33.3%)	10 (66.7%)	4 (26.7%)	11 (73.3%)	6 (40%)	9 (60%)
Symptoms							
No	29 (40.8%)	3 (10.3%)	26 (89.7%)	8 (27.6%)	21 (72.4%)	16 (55.2%)	13 (44.8%)
Mild	4 (5.6%)	0 (0%)	4 (100%)	1 (25%)	3 (75%)	1 (25%)	3 (75%)
Moderate	27 (38.0%)	3 (11.1%)	24 (88.9%)	4 (14.8%)	23 (85.2%)	7 (25.9%)	20 (74.1%)
Severe	11 (15.5%)	5 (45.5%)	6 (54.5%)	2 (18.2%)	9 (81.8%)	1 (9.1%)	10 (90.9%)
Sinus tract							
Yes	13 (18.3%)	3 (23.1%)	10 (76.9%)	2 (15.4%)	11 (84.6%)	2 (15.4%)	11 (84.6%)
No	58 (81.7%)	8 (13.8%)	50 (86.2%)	13 (22.4%)	45 (77.6%)	23 (39.7%)	35 (60.3%)
Lesion size							
<5 mm (small)	15 (21.1%)	1 (6.7%)	14 (93.3%)	4 (26.7%)	11 (73.3%)	8 (53.3%)	7 (46.7%)
5–10 mm (large)	37 (52.1%)	5 (13.5%)	32 (86.5%)	4 (10.8%)	33 (89.2%)	11 (29.7%)	26 (70.3%)
≥10 mm (too large)	19 (26.8%)	5 (26.3%)	14 (73.7%)	7 (36.8%)	12 (63.2%)	6 (31.6%)	13 (68.4%)
Apical limit of filling							
Overfill	9 (12.7%)	2 (22.2%)	7 (77.8%)	6 (66.7%)	3 (33.3%)	6 (66.7%)	3 (33.3%)
0–2 mm short	52 (73.2%)	8 (15.4%)	44 (84.6%)	9 (17.3%)	43 (82.7%)	19 (36.5%)	33 (63.5%)
>2 mm short	10 (14.1%)	1 (10%)	9 (90%)	0 (0%)	10 (100%)	0 (0%)	10 (100%)
Quality of root canal treatment							
Adequate	59 (83.1%)	10 (16.9%)	49 (83.1%)	15 (25.4%)	44 (74.6%)	25 (42.4%)	34 (57.6%)
Inadequate	12 (16.9%)	1 (8.3%)	11 (91.7%)	0 (0%)	12 (100%)	0 (0%)	12 (100%)
Quality of coronal restoration							
Adequate	45 (63.4%)	8 (17.8%)	37 (82.2%)	12 (26.7%)	33 (73.3%)	17 (37.8%)	28 (62.2%)
Inadequate	10 (14.1%)	2 (20%)	8 (80%)	1 (10%)	9 (90%)	5 (50%)	5 (50%)
Absent	16 (22.5%)	1 (6.3%)	15 (93.8%)	2 (12.5%)	14 (87.5%)	3 (18.8%)	13 (81.2%)
Histopathologic diagnosis							
Abscess (nonepithelialized)	16 (22.5%)	2 (12.5%)	14 (87.5%)	3 (18.8%)	13 (81.3%)	9 (56.3%)	7 (43.7%)
Abscess (epithelialized)	5 (7%)	1 (20%)	4 (80%)	1 (20%)	4 (80%)	1 (20%)	4 (80%)
Cyst (bay)	12 (16.9%)	7 (58.3%)	5 (41.7%)	6 (50%)	6 (50%)	4 (33.3%)	8 (66.7%)
Cyst (true)	1 (1.4%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Granuloma (nonepithelialized)	30 (42.3%)	1 (3.3%)	29 (96.7%)	4 (13.3%)	26 (86.7%)	10 (33.3%)	20 (66.7%)
Granuloma (epithelialized)	7 (9.9%)	0 (0%)	7 (100%)	1 (14.3%)	6 (85.7%)	1 (14.3%)	6 (85.7%)

Bold face, $P < .05$.**Campello:** Formal analysis, Software. **Irina****Milovidova:** Visualization, Investigation.**Isabela N. Rôças:** Visualization, Formalanalysis. **José F. Siqueira:**

Conceptualization, Methodology, Writing – original draft.

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*Domenico Ricucci: Conceptualization, Methodology, Investigation. Andrea F.**Campello: Formal analysis, Software. Irina**Milovidova: Visualization, Investigation.**Isabela N. Rôças: Visualization, Formal**analysis. José F. Siqueira Jr:**Conceptualization, Methodology, Writing–**Original draft preparation.**The authors deny any conflicts of interest related to this study.*

TABLE 3 - Prevalence of Extraradicular Infection in Teeth with Post-treatment Apical Periodontitis According to Clinical Features and Histopathologic Diagnosis

Variables	n = 71	Biofilm		Calculus		Bacteria in the cyst lumen*		Planktonic cells or flocs in the lesion body	
		Absent	Present	Absent	Present	Absent	Present	Absent	Present
Total	71 (100%)	48 (67.6%)	23 (32.4%)	65 (91.5%)	6 (8.5%)	9 (69.2%)	4 (30.8%)	46 (64.8%)	25 (35.2%)
Specimen obtained by									
Extraction	29 (40.8%)	25 (86.2%)	4 (13.8%)	28 (96.6%)	1 (3.4%)	2 (66.7%)	1 (33.3%)	26 (89.7%)	3 (10.3%)
Apicoectomy	42 (59.2%)	23 (54.8%)	19 (45.2%)	37 (88.1%)	5 (11.9%)	7 (70%)	3 (30%)	20 (47.6%)	22 (52.4%)
Sex									
Male	35 (49.3%)	20 (57.1%)	15 (42.9%)	31 (88.6%)	4 (11.4%)	4 (50%)	4 (50%)	20 (57.1%)	15 (42.9%)
Female	36 (50.7%)	28 (77.8%)	8 (22.2%)	34 (94.4%)	2 (5.6%)	5 (100%)	0 (0%)	26 (72.2%)	10 (27.8%)
Age (y)									
≤25	20 (28.2%)	17 (85%)	3 (15%)	18 (90%)	2 (10%)	2 (100%)	0 (0%)	14 (70%)	6 (30%)
26–40	24 (33.8%)	15 (62.5%)	9 (37.5%)	23 (95.8%)	1 (4.2%)	4 (66.7%)	2 (33.3%)	16 (66.7%)	8 (33.3%)
≥40	27 (38.0%)	16 (53.3%)	11 (40.7%)	24 (88.9%)	3 (11.1%)	3 (60%)	2 (40%)	16 (59.3%)	11 (40.7%)
Intervention									
Treatment	56 (78.9%)	42 (75%)	14 (25%)	52 (92.9%)	4 (7.1%)	6 (75%)	2 (25%)	40 (71.4%)	16 (28.6%)
Retreatment	15 (21.1%)	6 (40%)	9 (60%)	13 (86.7%)	2 (13.3%)	3 (60%)	2 (40%)	6 (40%)	9 (60%)
Symptoms									
No	29 (40.8%)	26 (89.7%)	3 (10.3%)	28 (96.6%)	1 (3.4%)	3 (100%)	0 (0%)	25 (86.2%)	4 (13.8%)
Mild	4 (5.6%)	3 (75%)	1 (25%)	4 (100%)	0 (0%)	0 (0%)	1 (100%)	2 (50%)	2 (50%)
Moderate	27 (38%)	14 (51.9%)	13 (48.1%)	25 (92.6%)	2 (7.4%)	4 (57.1%)	3 (42.9%)	16 (59.3%)	11 (40.7%)
Severe	11 (15.5%)	5 (45.5%)	6 (54.5%)	8 (72.7%)	3 (27.3%)	2 (100%)	0 (0%)	3 (27.3%)	8 (72.7%)
Sinus tract									
Yes	13 (18.3%)	3 (23.1%)	10 (76.9%)	8 (61.5%)	5 (38.5%)	3 (75%)	1 (25%)	5 (38.5%)	8 (61.5%)
No	58 (81.7%)	45 (77.6%)	13 (22.4%)	57 (98.3%)	1 (1.7%)	6 (66.7%)	3 (33.3%)	41 (70.7%)	17 (29.3%)
Lesion size									
<5 mm (small)	15 (21.1%)	14 (93.3%)	1 (6.7%)	15 (100%)	0 (0%)	0 (0%)	0 (0%)	14 (93.3%)	1 (6.7%)
5–10 mm (large)	37 (52.1%)	31 (83.8%)	6 (16.2%)	35 (94.7%)	2 (5.4%)	5 (71.4%)	2 (28.6%)	26 (70.3%)	11 (29.7%)
≥10 mm (too large)	19 (26.8%)	3 (15.8%)	16 (84.2%)	15 (78.9%)	4 (21.1%)	4 (66.7%)	2 (33.3%)	6 (31.6%)	13 (68.4%)
Apical limit of filling									
Overfill	9 (12.7%)	3 (33.3%)	6 (66.7%)	8 (88.9%)	1 (11.1%)	1 (33.3%)	2 (66.7%)	3 (33.3%)	6 (66.7%)
0–2 mm short	52 (73.2%)	36 (69.2%)	16 (30.8%)	47 (90.4%)	5 (9.6%)	6 (85.7%)	1 (14.3%)	35 (67.3%)	17 (32.7%)
>2 mm short	10 (14.1%)	9 (90%)	1 (10%)	10 (100%)	0 (0%)	2 (66.7%)	1 (33.3%)	8 (80%)	2 (20%)
Quality of treatment									
Adequate	59 (83.1%)	40 (67.8%)	19 (32.2%)	54 (91.5%)	5 (8.5%)	7 (70%)	3 (30%)	39 (66.1%)	20 (33.9%)
Inadequate	12 (16.9%)	8 (66.7%)	4 (33.3%)	11 (91.7%)	1 (8.3%)	2 (66.7%)	1 (33.3%)	7 (58.3%)	5 (41.7%)
Quality of coronal restoration									
Adequate	45 (63.4%)	26 (57.8%)	19 (42.2%)	39 (86.7%)	6 (13.3%)	6 (66.7%)	3 (33.3%)	26 (57.8%)	19 (42.2%)
Inadequate	10 (14.1%)	8 (80%)	2 (20%)	10 (100%)	0 (0%)	1 (100%)	0 (0%)	6 (60%)	4 (40%)
Absent	16 (22.5%)	14 (87.5%)	2 (12.5%)	16 (100%)	0 (0%)	2 (66.7%)	1 (33.3%)	14 (87.5%)	2 (12.5%)
Histopathologic diagnosis									
Abscess (nonepithelialized)	16 (22.5%)	6 (37.5%)	10 (62.5%)	15 (93.8%)	1 (6.3%)	0 (0%)	0 (0%)	3 (18.8%)	13 (81.3%)
Abscess (epithelialized)	5 (7%)	3 (60%)	2 (40%)	3 (60%)	2 (40%)	0 (0%)	0 (0%)	3 (60%)	2 (40%)
Cyst (bay)	12 (16.9%)	5 (41.7%)	7 (58.3%)	11 (91.7%)	1 (8.3%)	8 (66.7%)	4 (33.3%)	6 (50%)	6 (50%)
Cyst (true)	1 (1.4%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)
Granuloma (nonepithelialized)	30 (42.3%)	27 (90%)	3 (10%)	29 (96.7%)	1 (3.3%)	0 (0%)	0 (0%)	27 (90%)	3 (10%)
Granuloma (epithelialized)	7 (9.9)	6 (85.7%)	1 (14.3%)	6 (85.7%)	1 (14.3%)	0 (0%)	0 (0%)	6 (85.7%)	1 (14.3%)

Bold face, $P < .05$.*Prevalence data among the cysts only ($n = 13$).

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