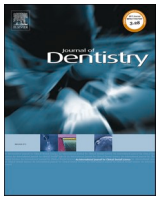
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Clinical and GCF outcomes of Minimally-invasive non-surgical periodontal therapy in teeth with bone loss to the apex. A prospective study

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ABSTRACT

Aims: The aim of this prospective study was to assess the potential benefits of minimally-invasive non-surgical therapy (MINST) in the healing of intrabony defects reaching the apex.
Methods: Twenty systemically healthy patients with stage III-IV periodontitis and with at least one tooth with an intrabony defect reaching the apex were included in the study. Step 1 and 2 periodontal therapy including MINST were provided to all patients and patients were then followed up to 12 months post-treatment. Clinical parameters, standardised periapical radiographs and gingival crevicular fluid (GCF) samples were collected at test and control sites. 31 biomarkers were analysed.
Results: Three cases did not have the 12-months review (2 drop-outs and 1 tooth lost due to fracture). Despite significant improvements in clinical and radiographic parameters, the majority of defects still had PPD>5mm at the 12-months reassessment, therefore needing further treatment, including endodontic treatment in some cases. A pattern of increase in GCF biomarkers associated with a senescent secretory phenotype was detected immediately after treatment in test sites, with a decrease up to 12 months. An increase in IL-1β and MMP-3 in particular seems to be associated with increased risk to need endodontic treatment post-MINST.
Conclusions: The treatment of teeth with unfavourable prognosis and bone loss to the apex with MINST leads to PPD reduction, CAL gain and reduction in radiographic defect depth 12 months after treatment. However, MINST alone may not be sufficient in terms of defects resolution in most of these cases.
Clinical significance: The treatment of teeth with unfavorable prognosis and bone loss to the apex (hopeless) using MINST may lead to consistent PPD reduction, CAL gain and radiographic defect depth reduction after 12 months. However, adjunctive therapies may be necessary to achieve endpoints of therapy in these defects. GCF biomarkers may clarify healing patterns.

1. Introduction

'Intrabony' or vertical periodontal defects are associated with higher risk of progression compared with sites affected by suprabony (horizontal) defects [1]. Intrabony periodontal attachment and bone loss can reach such advanced levels as to compromise tooth retention. Teeth which are thought not to respond to treatment have been referred to as 'hopeless'. Various definitions of hopeless have been used, ranging from loss of over 75% supporting bone, PPDs > 8 mm, furcation and mobility grade III [2], loss of $\geq 50\%$ bone height at either of the proximal sites, or class III furcation involvement [3] or a more generic 'inadequate attachment to maintain the tooth' [4]. The notion of 'hopeless' was challenged in the last decade and it was shown that regenerative therapy associated with endodontic therapy was effective at retaining 'hopeless' teeth with deep vertical bone loss ('intrabony defects') to the apex up to 5 years [5].

A handful of studies based on the concept of minimally-invasive non-surgical therapy (MINST), has shown the possibility to achieve pocket reduction and radiographic bone gain in teeth affected by deep intrabony defects [6,7] and that this reduction is stable long-term [8]. MINST is a type of non-surgical periodontal therapy which follows a detailed protocol based on specific principles of minimal invasiveness. The effect of MINST may be mediated by improved blood flow and stable blood clot in the intrabony defect, associated with elimination of inflammation. Although anecdotal cases of clinical and radiographic resolutions by MINST in teeth with furcation involvement and bone loss to the apex

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have been observed [9], no study to our knowledge has tested the efficacy of MINST in the healing of teeth with bone loss to the apex. Therefore, the aim of this study was to assess the clinical and radiographic outcomes and healing patterns following MINST in teeth with intrabony defects to the apex.

2. MATERIALS and methods

2.1. Patient population

This was a pilot proof-of principle prospective, single-centre cohort study to assess the effect of a modified MINST protocol in the healing of teeth with unfavourable periodontal prognosis (teeth with bone loss to the apex). Twenty consecutive periodontitis patients referred by their general dental practitioners to Guy's Hospital for periodontal treatment were included in this study. Ethics approval for the analysis was given by the London Bloomsbury Research Ethics Committee (reference IRAS number 265,686). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed, available in the Supplemental material 1. The study was registered on clinicaltrials.gov (reference NCT04236648). Each patient gave written consent to take part in the study. Patient visits took place from September 2020 to April 2023. The following inclusion criteria were considered for patient recruitment:

- Age 18–70
- Diagnosis of Periodontitis stage III or IV (grade A, B or C) [10]
- Presence of teeth with unfavourable prognosis (also defined as 'hopeless' by Cortellini et al. [11]), defined as having:
  A. radiographic bone loss to the apex or beyond the apex on one inter-dental aspect
  B. presence of severe attachment loss (>10 mm) at the same interproximal site/s and at least at one of either the buccal or lingual aspect [12]
  C. lack of function because of hypermobility, and/or lack of chewing strength, and/or pain, and/or recurrent periodontal infection/abscesses [12]
  D. positive response to vitality testing with electric pulp test (EPT) or previous endodontic treatment considered satisfactory, as judged by the examining clinician
- Willing to sign informed consent to take part in the study

Exclusion Criteria were: i) Smoking (any current or in past 5 years), ii) Medical history including diabetes or hepatic or renal disease, or other serious medical conditions or transmittable diseases, iii) History of conditions requiring prophylactic antibiotic coverage prior to invasive dental procedures, iv) Anti-inflammatory or anticoagulant therapy during the month preceding the baseline exam, v) Systemic antibiotic therapy during the 3 months preceding the baseline exam, vi) History of alcohol or drug abuse, vii) Self-reported pregnancy or lactation, viii) Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that according to the investigator may increase the risk associated with trial participation, ix) Periodontal treatment to the study site within the last 12 months.

3. Clinical examination

Following consent, at baseline, self-reported patient medical and smoking histories were checked. The following periodontal measurements were taken at six sites/tooth: dichotomous full mouth plaque scores (FMPS) [13], full mouth probing pocket depth (PPD), recession (REC) of the gingival margin from the cemento-enamel junction (CEJ), bleeding on probing (FMBS) [14], tooth mobility [15] and furcation involvement [16]. Clinical attachment level (CAL) was calculated as PPD+REC. Clinical parameters were assessed by gentle probing using a UNC-15 periodontal probe and a Nabers probe for furcation involvement. The study 'Test site' was chosen as the interproximal site of the tooth falling into the 'unfavourable' definition above. If more than one tooth with that definition was detected in each patient, the site with the deepest PPD was selected. A 'Control site' was chosen among healthy sites in the contralateral arch, showing PPD <4 mm and no BOP.

4. Periodontal treatment and follow-ups

The study flowchart is presented in Fig. 1. All patients received a comprehensive treatment plan, aimed at step 1 and step 2 periodontal therapy [17], including oral hygiene instructions, risk factor control and supra-gingival scaling. Sub-gingival scaling was carried out in 1 to 2 sessions, following a previously-described modified MINST protocol [8]. Patients were then reassessed at day 1, day 7, 1 month, 3 months, 6 months, 9 months and 12 months post-treatment. At these appointments, patients' medical and dental history were reviewed, adverse events and concomitant medications were checked, oral hygiene measures were checked and re-enforced and supra-gingival debridement was carried out. Sites found to require sub-gingival debridement or other forms of treatment were treated according to clinical needs. At the 12 months appointment, a new treatment plan was carried out.

4.1. Examiner calibration

Two calibrated examiners (authors JM and PM) carried out periodontal assessments. Intra- and inter-examiner training and calibration exercises showed more than 95% agreement of PPD and CAL within ± 2 mm in double measurements of 5 non-study patients with at least a 15-minute gap. Lin's concordance correlation coefficient for PPD for examiner 1, examiner 2 and inter-examiners were 0.96, 0.94 and 0.71 respectively.

4.2. Radiographs

A standardized periapical radiograph of selected study sites was taken at baseline and at the 6-month and 12-month follow-up visits following MINST with a customised holder and an occlusal platform, which allowed a cold-cure acrylic resin occlusal registration to be made (bite index), facilitating relocation of the holder and preserving the projection geometry in subsequent radiographs. Impressions for standardised radiographs were taken at baseline. Aluminium step wedges were used as a densitometric reference, in an attempt to minimize errors due to variation in exposure time and/or film processing which may result in false positive analysis [18]. Periapical radiographs from all patients included in the study were screened, entered in a dedicated database, given a masked code, transferred into a dedicated software system (Xposeit version 3.01, Torben Jørgensen, Lystrup, Denmark) and analysed blindly by one designated examiner (author PM) as described before [19] at all comparable sites (mesial and distal) to calculate percentage of bone loss by root length. Lin's concordance correlation coefficient for radiographic defect depth following their radiographic calibration exercise (double measurements of intrabony defects on the radiographs of 10 non-study patients) was 0.99.

4.3. Gingival crevicular fluid sampling and analysis

Samples of GCF were collected from the selected intrabony and control sites at baseline and at the day 1, day 7, 1 month, 6 months and 12 months following MINST, prior to periodontal probing to avoid contamination by blood. Saliva was removed from the supra-gingival area using saliva ejector and cotton rolls, being careful not to touch the gingival margin area; supra-gingival plaque, if present, was removed using a curette to prevent saliva and/or plaque contamination. GCF was collected for 30 s using methylcellulose strips carefully placed gently at the entrance of the sulcus until slight resistance was felt. GCF volume was measured by Periotron, after which samples were placed on ice

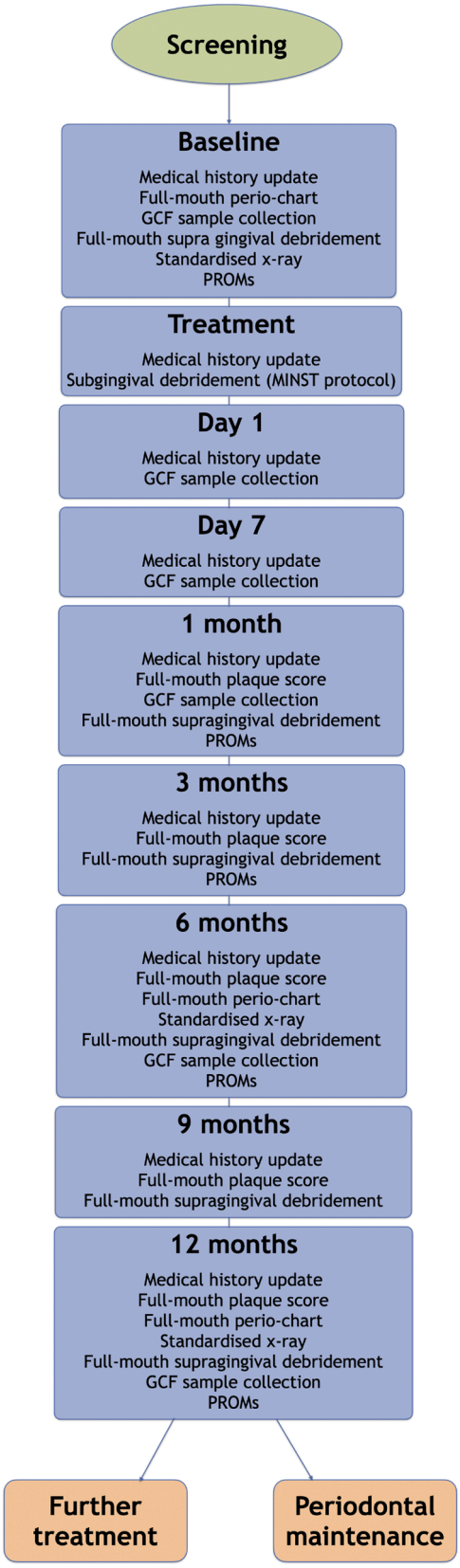


Fig. 1. Study flowchart.

before being transferred to a laboratory freezer for storage at −70 °C for storage and analysis at the end of the study. Within 2 weeks of sampling, GCF was extracted in acidic buffer to better preserve inflammatory mediators of periodontal disease from breakdown and/or oxidative processes. GCF was eluted and processed for a bead-based multiplex immunoassays (Luminex, R&D systems, Minneapolis, MN, USA) as described before [20] and in supplemental material 2. Thirty-one analytes were selected according to previous literature as inflammatory, tissue degradation and repair/regenerative markers. They can be broadly divided into [21]:

- 'inflammation biomarkers': interferon-gamma (IFN-γ), interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin- 8 (IL-8), interleukin-10 (IL-10), interleukin-17 (IL-17), lactoferrin, E-selectin, tumor necrosis factor-alpha (TNF-α) and CCL2
- 'tissue degradation biomarkers': aggrecan, matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-3 (MMP-3), matrix metalloproteinase- 8 (MMP-8), tissue inhibitor metalloproteinase-1 (TIMP-1)
- 'repair/regeneration biomarkers': angiopoietin-1 (Ang-1), bone morphogenic protein-2 (BMP-2), epidermal growth factor (EGF), basic fibroblast growth factor (FGFb), osteopontin (OPN), platelet-derived growth factor-AA (PDGF-AA), derived growth factor-BB (PDGF-BB), platelet-derived growth factor-AB (PDGF-AB), periostin, vascular endothelial growth factor (VEGF), acidic fibroblast growth factor (FGFa), osteoprotegerin (OPG), and sclerostin (SOST).

4.4. Patient-reported outcome measures (PROMs)

PROMs were collected using the OHIP-14 questionnaire [22] at baseline, 1-, 3-, 6- and 12-months following MINST, assessing 7 domains: functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability and handicap.

4.5. Statistical analysis and sample size calculation

This was a pilot proof-of-principle study, using a convenience sample of 20 sites in 20 patients. The primary outcome of the study was the radiographic defect change (in mm) between baseline and at 12 months. Secondary outcomes were CAL and PPD changes between baseline and 12 months, inflammatory markers and growth factors in gingival crevicular fluid (GCF) and saliva, bacterial detection associated with presence of intrabony defects and patient-reported outcomes.

Data from all patients were entered into a spreadsheet by independent staff and proofed for entry errors. Paired *t*-test was used to detect significant changes between baseline and 12 months for intrabony defect depth (primary outcome), PPD, CAL, PROMs and GCF volume using the patient as unit of analysis. Descriptive data was presented by pre-specified prognostic baseline factors (age, FMPS, BMI, CAL).

GCF biomarker output concentration (pg/ml) output from the Luminex data was tested for normality using the Shapiro-Wilk test. The GCF biomarker data were summarised as medians and interquartile ranges. The difference between the healthy and intrabony sites were compared using the Wilcoxon matched pairs signed-ranks test as GCF biomarker data was not normally distributed. Further to this, a Wilcoxon test was done to compare the GCF biomarker differences between baseline and follow-up timepoints at test sites and for OHIP-14 scores. For exploratory analyses, the patients were then separated into a "success" group (defined as PPD reduction ≥3 mm and CAL gain ≥3 mm) and a "no success" group. A further exploratory analysis was based on whether the test tooth was deemed to require endodontic intervention during or at completion of the study ('endo need'). Differences in these biomarkers were then compared via the Mann Whitney U test. Due to multiple analytes being investigated, a Bonferroni correction was done

to counteract multiple testing resulting in a significance level $\alpha = 0.05/31 = 0.0016$.

5. Results

5.1. Baseline characteristics

Twenty patients entered the study. The average age of the patients was 51 years, 60% were female, 45% were of Caucasian ethnicity and 40% were former smokers (Table 1). They had an average of 26 teeth present and an average of 30 sites with PPDs >4mm. The majority of study sites (80%) were located on multi-rooted teeth (Table 2). The average PPD was 11.7 mm, recession was 2 mm and CAL was 13.6 mm. Radiographically, the average depth of the suprabony and intrabony components were both 6.1 mm (total bony defect depth 12.2 mm) and the average defect angle was 35.6° The average treatment time per test tooth was 12.3 min.

All 20 teeth were still present at the 6-month reassessment and no adverse events were reported. However, in addition to 2 drop-outs (patients unable to attend the appointments), one tooth fractured after the 6 months appointment, resulting in 17 test teeth reassessed at 12 months. The radiographic total defect depth reduction (including only patients who attended the 12-months visits) was 1.52 ± 4.31 mm at 6 months and 1.98 ± 2.98 mm at 12 months, respectively ($p < 0.001$ compared with baseline). The radiographic intrabony component reduction (including only patients who attended the 6- and 12-months visits) was 0.67 ± 2.17 mm at 6 months and 1.50 ± 3.38 mm at 12 months, respectively ($p < 0.001$ compared with baseline). The average PPD reduction (including only patients who attended the 6- and 12-months visits) was 2.75 ± 2.29 mm at 6 months and 3.70 ± 3.04 mm at 12 months respectively ($p < 0.001$ compared with baseline). CAL gain was 2.05 ± 1.99 mm at 6 months and 2.94 ± 3.58 mm at 12 months ($p < 0.001$ compared with baseline). Recession increase was 0.7 mm at 6 months and at 12 months ($p = 0.009$ and $p = 0.017$ respectively) (Table 2).

Fig. 2 shows clinical and radiographic outcomes at 12 months of a site showing favourable outcome (Fig. 2A and B) and one showing unfavourable outcomes (Fig. 2C and D). A total of 7 out of 20 and 7 out of 17 sites achieved the definition of clinical 'success' at 6 months and 12 months respectively. None of the baseline patient, site and treatment variables were associated with 'success' at 6 and 12 months by logistic regression (supplemental material 3).

5.2. Endodontic conditions

Two test teeth had received root canal treatment before entering the study. Sensibility testing was carried out in all other study sites at each timepoint. Root canal treatment was carried out on three teeth throughout the study and on three further cases upon study completion, due to lack of improvement and/or worsening of the periapical area after treatment. These teeth were defined as 'endo need' for the analyses.

Table 1
Subject and clinical characteristics at baseline. (FMPS: full mouth plaque score. FMBS: full mouth bleeding score. BMI: Body Mass Index).

		Patients $n = 20$	%
Age		51.2 ± 8.6	
Gender	Male	8	40
	Female	12	60
Ethnicity	White	9	45
	Other/mixed	11	55
Smoking	Former smokers	8	40
	Never smoked	12	60
BMI		26.0 ± 3.7	
Number of teeth present		26.4 ± 3.2	
Number of pockets >4mm		30.3 ± 20.7	
FMPS		-	31.8 ± 11.8
FMBS		-	40.4 ± 17.9

5.3. PROMs

Supplemental material 4 shows results of PROMs analysis throughout the study. The total average OHIP-14 score per patient improved significantly between baseline and subsequent study visits. Most OHIP-14 domains improved at all study visits compared with baseline, and all except 'physical disability' and 'handicap' showed improvements at the last visit compared with baseline. Interestingly, although no associations were observed between OHIP-14 values and 'success', patients who ended up having to need endodontic treatment (in the 'endo need') had higher OHIP-14 scores at every timepoint (statistically significantly at the 3- and 6-months visits) (Table 3). Items in domains 'functional limitations' and 'physical pain' were particularly higher in patients with teeth which eventually needed endodontic treatment.

5.4. GCF analysis- Trend following treatment

All analytes were detected at baseline in intrabony sites. Table 4 shows comparisons of GCF analytes in intrabony sites compared with baseline. Most of them then increased 1 day post-treatment compared to baseline levels, including inflammatory markers (IL-1β, IL-4, IL-8, E-selectin, IL-2, IL-6, IFN-γ, TNF-a, CCL2 and IL-10), tissue degradation markers (aggrecan, MMP-1, MMP-3 and TIMP-1) and repair markers (periostin, VEGF, BMP-2, PDGF-AB, OPG and FGF-basic) ($p < 0.0016$) (Table 4). Only MMP-1, PDGF-AB and BMP-2 were still higher than baseline at day-7 ($p < 0.0016$) and PDGF-AB, BMP-2 and ANG-1 were higher than baseline at 1-month ($p < 0.0016$), showing a shift from mainly inflammatory and tissue degradation to mainly repair markers after day-1. A few analytes were higher than baseline at 6 months (lactoferrin, IFN-γ, PDGF-AA, VEGF and FGF-B) and at 12 months (IFN-γ).

5.5. GCF analysis- Test vs. control sites

Table 5 shows all studied GCF analytes, highlighting those increased in intrabony vs. control sites throughout the study visits. Intrabony sites had higher GCF volumes measured by Periotron compared with control sites at baseline, day-1, day-7 and 1 month follow-ups ($p < 0.001$), but not at 6 months ($p = 0.077$) and 12 months ($p = 0.103$) follow-ups. At baseline, intrabony sites had higher levels of inflammatory markers (IL-1β, IL-17, IL-6 and E-selectin), tissue degradation markers (MMP-1, MMP-8 and MMP-3) and repair markers (VEGF, FGF-basic and OPN) compared to healthy control sites ($p < 0.0016$). Most of the analytes across the three different groups (inflammatory, tissue degradation, repair) were higher at day 1 and day 7 compared with controls, while gradually fewer markers were still increased in intrabony vs. control sites at subsequent study visits. Supplemental material 5 shows the patterns of all analytes in intrabony and healthy sites across the study visits.

5.6. Exploratory analyses

No statistically significant differences were detected for Periotron values and for any of the GCF analytes at any timepoint between the 'success' and 'no success' groups.

Fig. 3 shows IL-1β and MMP-3 GCF levels throughout the study for cases divided by teeth needing endodontic treatment during or at completion of the study ('endo' group, $n = 6$) and other test teeth ($n = 10$). No differences in Periotron readings between groups were detected throughout the study. Although elevation in several biomarkers was detected at baseline and day 1 in the 'endo' teeth compared with those

Table 2
Comparison of clinical and radiographic parameters of the study sites between baseline and 6-month reassessment following MINST. Paired T-test p values for the differences between the two time-points are shown in the last columns. (PPD: Pocket probing depth, REC: recession, CAL: Clinical attachment level, CEJ: cemento-enamel junction).

	Teeth ($n = 20$)			*t*-test	
	Baseline (mean ± SD)	6-months re-evaluation (mean ± SD)	12-months re-evaluation	Baseline vs 6 M	Baseline vs 12 M
PPD (deepest site) (mm)	11.7 ± 2.8	8.9 ± 3.1	7.8 ± 2.9	$p < 0.001$	$P < 0.001$
REC (mm)	2.0 ± 1.9	2.6 ± 2.1	2.7 ± 2.1	$p = 0.009$	$P = 0.017$
CAL (mm)	13.6 ± 2.8	11.5 ± 3.4	10.5 ± 2.9	$p < 0.001$	$P = 0.001$
Total radiographic defect depth (mm)	12.1 ± 3.1	11.0 ± 3.8	10.2 ± 3.1	$p = 0.070$	$P = 0.005$
Suprabony defect depth (CEJ to bone crest) (mm)	6.1 ± 2.7	5.8 ± 3.0	5.8 ± 3.6	$p = 0.440$	$P = 0.397$
Infrabony defect depth (mm)	6.1 ± 3.5	5.2 ± 3.5	4.3 ± 3.1	$p = 0.090$	$P = 0.022$
Defect angle (°)	35.5 ± 14.3	48.1 ± 27.8	48.0 ± 21.8	$p = 0.022$	$P = 0.009$

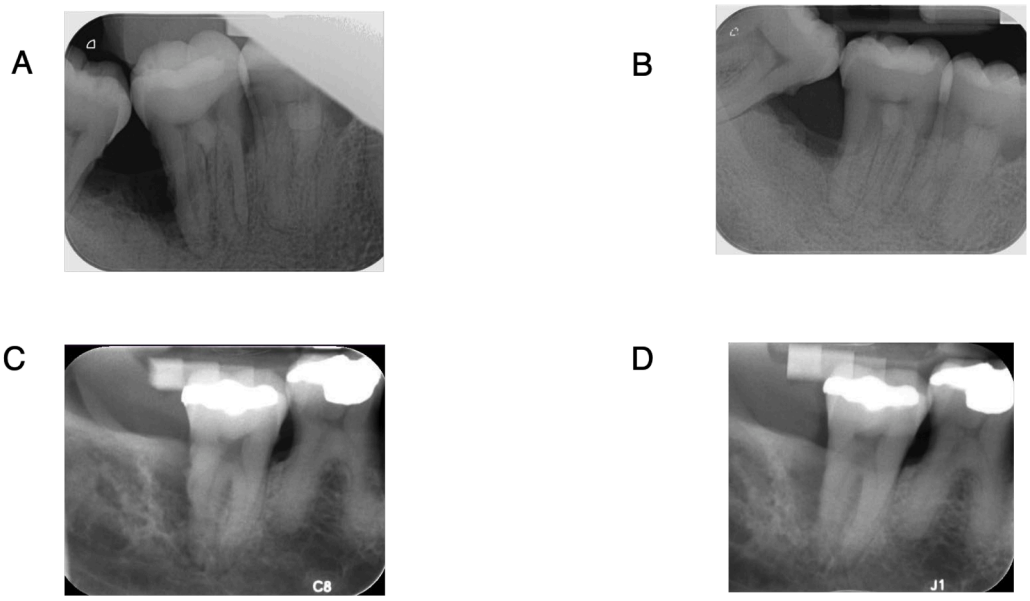


Fig. 2. Examples of baseline and 12-months radiographs (respectively A and B) of a case showing good reduction of clinical and radiographic disease parameters post-MINST, and a case showing unfavourable response (respectively C and D for baseline and 12-months follow-up).

Table 3
Average total OHIP-14 scores per patient divided by 'need for endo' and 'no need for endo' at each study visit, obtained by giving a score of '0′ to answer 'never' and incrementally until '4′ for 'very often'.

Visits	Need for endo ($n = 6$)	No need for endo ($n = 10$)	Mann-Whitney test $p=$
Baseline	24.3 ± 12.6	11.3 ± 7.9	0.073*
1 month	12.3 ± 7.9	6.8 ± 5.0	0.220**
3 months	14.1 ± 10.8	4.0 ± 3.9	0.016**
6 months	23.1 ± 15.5	5.5 ± 4.5	0.003^
12 months	14.0 ± 12.2	3.8 ± 4.0	0.073^^

* $=p < 0.05$ for domains 1 and 4.
** $p < 0.005$ for domains 1 and 2.
^ $p < 0.005$ for domains 1, 2 and 6.
^^ $p < 0.005$ for domain 1.

found to need endodontic treatment, these differences became statistically significant only at day-7 for IL-1β and MMP-3 ($p < 0.001$). MMP-3 was still statistically significantly higher in the 'endo' group at 1 month. Other markers including IL-1β, IL-8, MMP-1, MMP-8, TIMP-1, VEGF, periostin, OPN, FGF-B, IL-6 and E-selectin were also higher in 'endo' teeth compared with other teeth ($p < 0.05$), but differences did not reach the stricter statistical significance threshold of $p < 0.016$. No statistically significant differences were detected at 6 months and 12 months.

6. Discussion

This proof-of principle study investigated the effect of MINST on teeth with intrabony defects to the apex, previously defined as 'hopeless' [5]. Twelve months post-treatment, the average PPD reduction was 4.44 mm, CAL gain was 1.75 mm, while radiographic bone gain was 2 mm on average. PPD reduction and radiographic bone gain achieved in this study are fairly similar to other studies investigating the outcomes of the treatment of intrabony defects treated with MINST [7,23,24]. However, due to the very severe initial periodontal conditions in these cases, almost all study teeth needed further treatment following study completion, as endpoints of therapy were achieved only in a few cases [25]. Therefore, this study suggests that, while it has been shown that MINST can significantly reduce the radiographic intrabony defect depth and PPD for teeth with bone loss to the apex [9], this seems to be highly unpredictable.

Interesting findings derive from the extensive investigation of GFC biomarkers throughout the study. At baseline, GCF volume was higher at intrabony sites compared to healthy sites, in line with an increase in GCF following gingival inflammation [26]. One day after treatment the GCF volume at the intrabony site increased, compatibly with the inflammatory phase of wound healing. Inflammation was higher in the intrabony

Table 4
GCF biomarkers divided by type. Grey cells indicate values statistically significantly higher ($p < 0.016$) compared with baseline value for each marker.

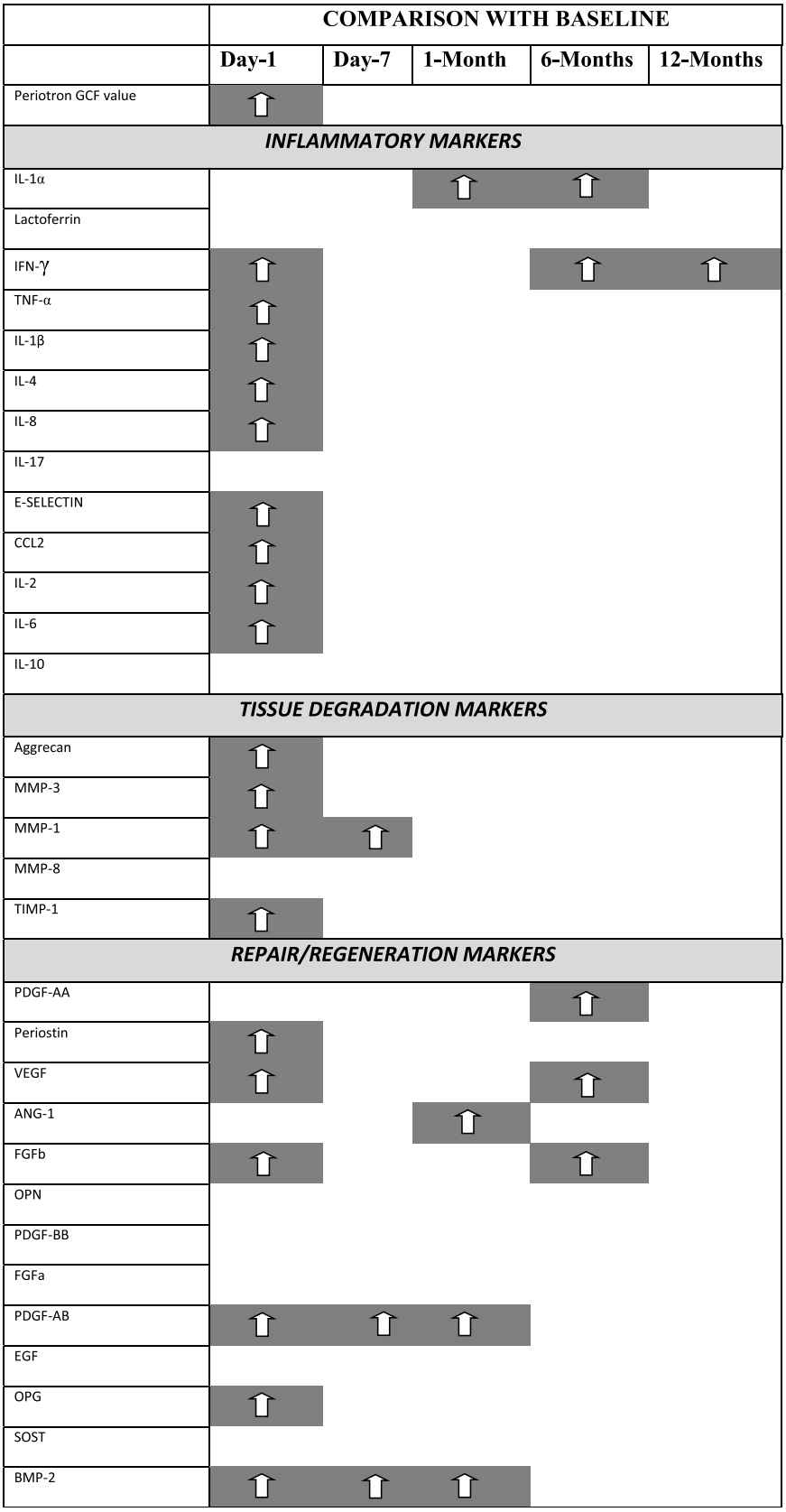
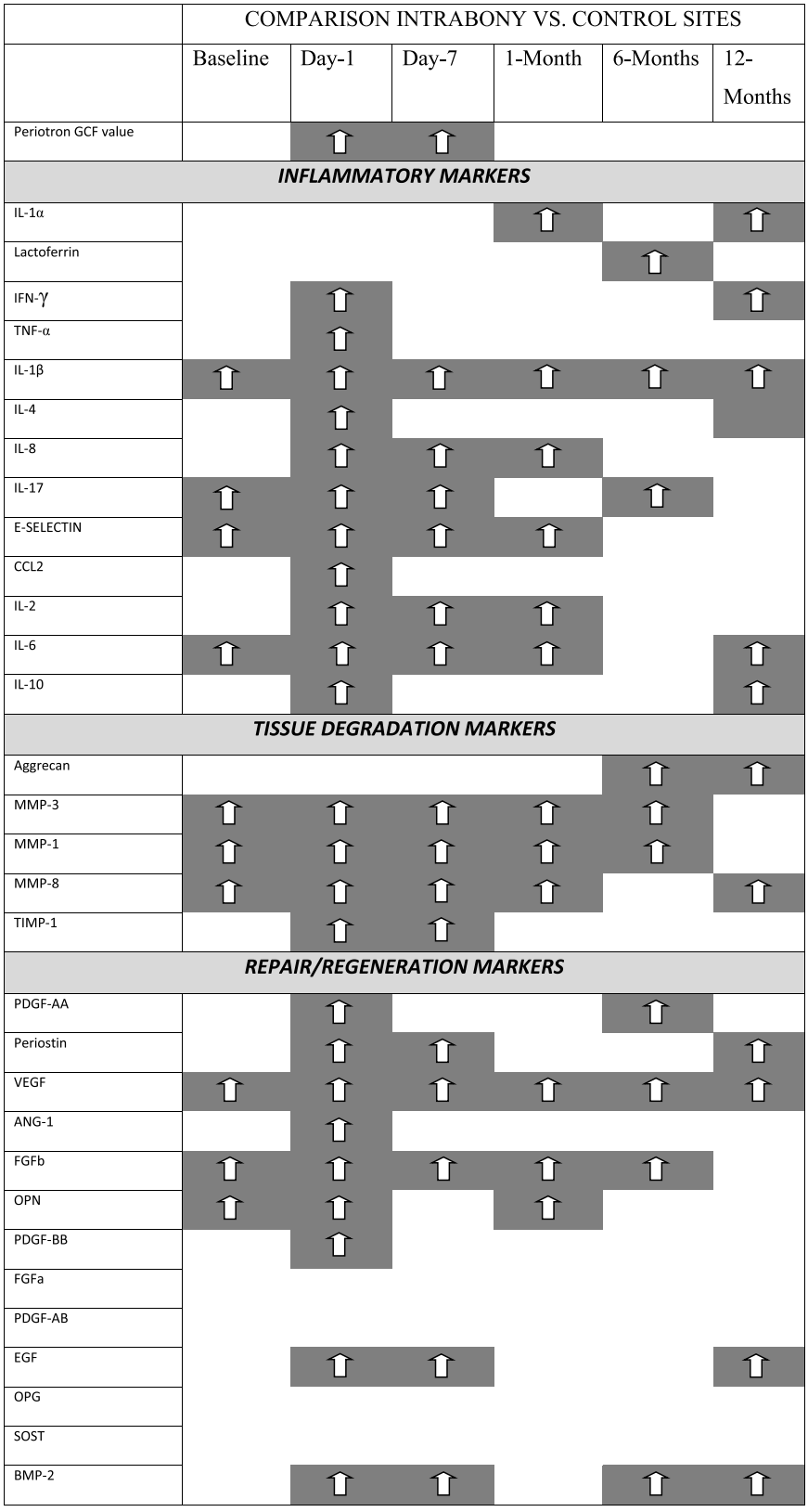


Table 5
GCF biomarkers divided by type. Grey cells indicate values statistically significantly higher ($p < 0.016$) compared with baseline value for each marker.



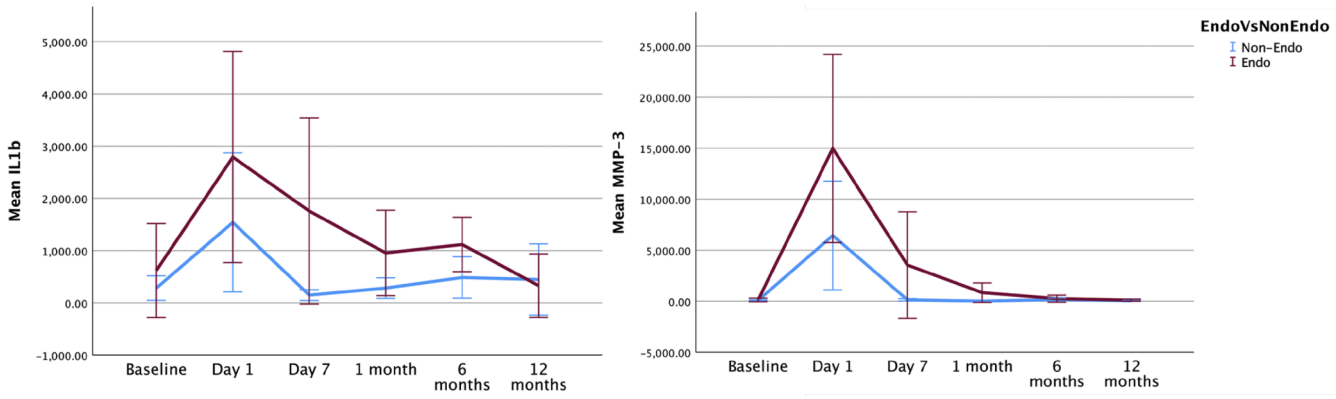


Fig. 3. IL-1β and MMP-3 GCF levels throughout the study for cases divided by teeth needing endodontic treatment during or at completion of the study ('endo' group, $n = 6$) and other test teeth ($n = 10$).

site compared to the healthy sites, as shown by biomarkers such as IL-1β, IL-6, IL-17 and IFN-γ, as expected in sites affected by periodontal inflammation [21,27-32]. The same pattern was followed by tissue degradation markers such as MMP-1, MMP-3 and MMP-8, and 'repair' markers such as OPG, VEGF and FGF-basic, in line with previous observations [21]. The increased amount of 'repair' markers pre-treatment in intrabony defects compared with healthy sites is a confirmation of previous findings [21], which have been extended also to suprabony defects [20]. The strong correlations between inflammatory, tissue degradation/remodelling and repair markers reflect the fact that periodontal wound healing is a complex interplay between inflammation, tissue remodelling and repair. Due to feedback and regulatory mechanisms, it is difficult to compartmentalise biomarkers in specific groups.

The finding that inflammatory markers increased initially and then decreased by 1 month post treatment supports the theory that inflammation and regeneration/repair are coupled in the wound healing process, with macrophages potentially being the bridge between the two [33]. These findings fit with the description of wound healing phases following periodontal therapy [34], with an initial increase in inflammatory markers at day 1, followed by a decrease by 1 month following treatment, in line with the initial inflammatory phase of healing which peters out by 1 month post treatment. While the inflammatory markers generally continued to decrease from day 7 to 1 month, some of the "repair" markers (FGF-B, VEGF, EGF, OPG) did not decrease or decreased less between these timepoints, corresponding to the remodelling phase of healing. A systematic review has recently shown that early healing following non-surgical periodontal therapy ($\leq$ 30 days) is characterised by an increase in pro-inflammatory cytokines, while at 6–8 weeks and up to 3 months IL-1β, IL-1, IL-6, TNF-α, IFN-γ, GM-CSF, MCP-1, and MIP-1α tended to reduce compared to baseline [35]. Data on surgical treatment of intrabony defects follows a similar pattern of an initial increase in PDGF-BB and VEGF [36] and PDGF-AB [37].

The GCF biomarker profile at this early phase of healing could be of great importance as it may sway the wound healing processes towards either regeneration or repair. In contrast to existing timelines on wound healing phases, the sharp increase in these repair markers at 1 day post treatment may indicate that intrabony defects express these markers earlier due to their capacity for healing via bony repair and even regeneration. However, there tended to be a sharp decrease in 'repair' markers at day-7 and 1 month following treatment, which could be due to the severity of these defects not lending themselves to repair/bony infill. It is also important to note that although angiogenesis is key to inflammation, the formation of new blood vessels is also a key part of the wound healing process, hence underlying the importance of VEGF in tissue repair and wound healing/regeneration following treatment [38]. MMP-8 has previously emerged as a GCF biomarker with high specificity and sensitivity for periodontitis [39]. It is the balance between MMPs and their inhibitors which may determine their destructive power. The present study shows a disruption to this balance at intrabony sites, where there is a higher amount of MMPs at baseline and post-treatment compared to their tissue inhibitors.

The GCF analysis can shed some light on treatment outcomes too. One of the reasons for the relatively less favourable clinical results compared with previous MINST studies may be that several of the study teeth, despite testing positive to sensibility testing pre-treatment, developed endodontic-periodontal lesions and/or apical pathology during the study. Six of the study teeth subsequently had to undergo root canal treatment during the 12-month follow-up or immediately following it. In some cases, endodontic treatment was initiated even when sensibility testing was still positive, due to an increase in the periapical radiolucency, suggesting that in some cases endodontic infections may have been present and latent before periodontal therapy. This may be due to extensive bone loss to or near to the apex, allowing bacteria to reach and start affecting the pulp through the apical foramen [40]. Any treatment of the periodontal pocket would have been less successful if pulpal involvement was present. Interestingly, teeth which eventually needed endodontic therapy showed a greater increase in some inflammatory, connective tissue degradation and repair biomarkers (particularly IL-1β and MMP-3) from 1 day to 1 month after treatment compared with sites which did not need endodontic treatment, despite similar GCF volumes. This may indicate an additional inflammatory process associated with the endodontic lesion. In some cases, non-surgical periodontal treatment of intrabony defects to the apex of the teeth may push the endodontic status of the tooth over the edge leading to loss of vitality of the tooth. Some sites may be more readily primed for an inflammatory response reflecting a communication between the endodontic and periodontal tissues. Alternatively, lack of resolution of inflammation post-treatment in these sites (persistence of high level of GCF inflammatory biomarkers after the 'inflammation phase') may have actually resulted in endodontic pathology. Therefore, in agreement with Cortellini et al. [5], elective root canal therapy may need to be considered for vital teeth with intrabony defects to and beyond the apex before periodontal treatment.

Another important finding in agreement with a previous report [21], is the characterisation of a senescence-associated secretory phenotype, with dysfunctional upregulation of IL-1β, IL-6, MMP-1 and MMP-3 at test sites compared to control which persisted after treatment in intrabony defects compared with healthy sites. Senescent cells are a powerful source of reactive oxygen species, proinflammatory factors and proteolytic enzymes which can exert a disruptive effect on the neighbouring cells and local environment. The sustained higher levels at the intrabony sites could suggest the presence of senescent cells which have been permanently altered to express these biomarkers, even following treatment. This is in agreement with a recent study, showing worse

clinical outcomes following regenerative periodontal therapy in the presence of elevated pre-treatment GCF levels of IL- 1β, MMP-8 and MMP-9 [41].

The clinical outcomes of this study may have been affected by the extreme severity of the lesions, the sub-clinical endodontic involvement in some cases, the lack of use of antimicrobial adjuncts and potentially the repeated disturbance of the healing process due to GCF samples collection. Despite this, the present study supports the use of MINST, due to its favourable characteristics promoting regeneration [42-44] and in improving clinical and radiographic outcomes [24,45-47]. PROMs measured with OHIP-14 improved from baseline to the other study visits, reflecting an improvement in oral-health related quality of life following treatment, in most domains except physical disability and handicap. It is very interesting to notice that patients with teeth which eventually needed endodontic therapy consistently reported worse OHIP-14 scores, particularly for 'functional limitations' and 'physical pain'. This is in parallel with the consistently increased GCF biomarkers in sites from these patients.

The primary strength of this study lies in its novelty as the first investigation on the effects of MINST specifically in a cohort of patients with teeth exhibiting an unfavourable prognosis accompanied by bone loss extending to the apex. It appears that MINST alone may not be enough to address and resolve these defects and that the majority of these defects would need adjuncts and/or subsequent surgical intervention to address the persisting deep pockets and vertical bony defects and achieve the endpoints of therapy [48]. The main limitation of this study is the absence of untreated controls is the main limitation of this study, which is partially mitigated by the use of control sites for the GCF analysis. Furthermore, many of the analyses presented are exploratory, and as such need to be interpreted with caution.

7. Conclusions

In conclusion, within the limitations of this study, the treatment of teeth with unfavourable prognosis and bone loss to the apex with MINST results in significant improvements in PPD reduction and CAL gain, and reduction in radiographic defect depth after 12 months. These teeth should be carefully and constantly monitored for pulpal involvement and apical pathology, and elective root canal treatment may be indicated. Analysis of patient-reported outcomes and of GCF markers associated with inflammation, connective tissue degradation and repair may provide further inside into healing pathways and potentially provide future opportunities for additional management options.

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CRediT authorship contribution statement

G Mainas: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **J Moore:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A Patel:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **P Menhadji:** Writing – review & editing, Methodology, Investigation, Data curation. **L Nibali:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

G Mainas and J Moore contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. A Patel and P Menhadji contributed to analysis and interpretation, and critically revised the manuscript. L Nibali contributed to conception, design, data analysis and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work. Authors G Mainas and J Moore equally contributed to this work.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jdent.2024.105403.

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