



Salivary and Gingival Biomarkers as Predictors of Orthodontic Tooth Movement: A Multi-Cohort Study.

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KEYWORDS

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ABSTRACT:

Orthodontic tooth movement (OTM) is a biologically mediated phenomenon characterized by bone remodelling, inflammatory response, and adaptability of periodontal tissues. Salivary and gingival biomarkers are of growing interest in reflecting the biological basis of orthodontic tooth movement. To assess the potential of salivary and gingival biomarkers in predicting orthodontic tooth movement based on a multi-cohort design. Multi-cohort study utilizing data from patients undergoing orthodontic treatment. Saliva samples and gingival crevicular fluid obtained at predetermined time intervals. Analysis of salivary biomarkers and gingival reflectors includes inflammatory mediators, enzymes related to tissue integrity, and bone remodelling markers. Rate of orthodontic tooth movement & treatment dynamics is correlated with expression profiles of salivary and gingival biomarkers, across multiple cohorts. 1,164 treatment records & datasets were assessed with 34 cohorts available for study. Levels of inflammatory cytokines such as IL-1 β , TNF- α , and prostaglandin E2 associated with higher rates of movement. Higher activity of other matrix remodelling enzymes and bone remodelling markers associated with treatment character. Customizable paradigms with precise biomarker profiles can predict movement & treatment efficiency across cohorts.

1. Introduction

There is significant complexity and strict regulation involved in orthodontic tooth movement (OTM)—these processes are highly interdependent, and therefore involve the mechanical forces placed on the teeth, to generate a cellular response within the periodontal ligament and alveolar bone. When orthodontic forces are applied to the teeth, a cascade of events will take place that is due to the aseptic nature of the inflammation, activation of osteoclasts mediating the resorption of the bone on the pressure side of the tooth, and the osteoblasts that are responsible for bone formation on the tension side of the tooth. Together these three cellular processes will culminate in the directional movement of the tooth. As the field of orthodontic biomechanics continues to advance, some of the same clinical parameters are still used today in orthodontic treatment—the force that is being applied, the design of the appliance being utilized in the treatment, and the planning of the treatment—however none of these parameters alone can predict the variability in the response that can be expected from the individual

patient. There is considerable heterogeneity that exists in the rate of the movement of the teeth that are undergoing orthodontic treatment, in the susceptibility to root resorption from orthodontic treatment (once again, an issue of heterogeneity), as well as in the long-term stability of the final outcome of the orthodontic treatment. In light of this, across the last two decades research into salivary and gingival crevicular fluid biomarkers have been investigated with the objective of determining their potential as non-invasive tools to assess real-time biological events taking place during OTM processes. According to research the use of biomarkers in this manner can provide dentists with mechanistic insight into the inflammatory, catabolic and anabolic pathways that underlie the OTM process; therefore allowing for dentists to personalize their treatment of patients by identifying the rate at which their teeth will be moved, to identify patients that are at risk for complications as a result of the orthodontic treatment, and to optimize the use of orthodontic interventions for each patient. [1]



2. Study Design and Methodology

Multi-Cohort Study Design

The study was developed as a multi-cohort design that is both longitudinal and prospective, and to comprise of three clinical research sites to examine biological variation in OTM by utilizing multiple cohorts separated by both age and periodontal condition. The total timeframe for the study will consist of a 24-month active treatment phase and a 12-month retention phase, so that the long-term outcome of the treatment can be assessed along with the potential for relapse. A total of three groups were created for this study: adolescent patients (aged 12 to 18), adult patients (aged 25 to 40) who do not have risk factors for periodontal disease, and adult patients (aged 30 to 55) with documented histories of gingivitis or mild to moderate periodontitis. These three groups allowed for comparison of kinetics of biomarkers as they relate to age-dependent remodelling potential and periodontal status. [2]

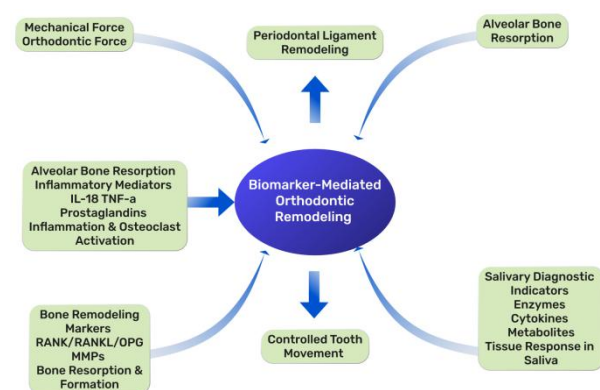


Figure 1. Biological Framework of Salivary and Gingival Biomarkers in Orthodontic Tooth Movement.

A general schematic was designed to present the role of specific biomolecules in mediating biological processes related to orthodontic movement, centred on the node Biomarker-Mediated Orthodontic Remodelling (depicted in dark blue). This node breaks down into three major domains: inflammatory mediators (IL-1 β , TNF- α , prostaglandins that stimulate localised inflammation and osteoclast activity), bone remodelling markers (RANK/RANKL/OPG), and saliva-based diagnostic indicators (enzymes, cytokines, and metabolites that reflect the tissue response to orthodontic forces). Solid arrows indicate that application of mechanical forces to teeth results in activation of biomarkers that lead to remodelling of the periodontal ligament, resorption of alveolar bone, and controlled movement of teeth through orthodontic treatment. [3]

Inclusion and Exclusion Criteria

To ensure reliability in this study, strict inclusion/exclusion criteria were employed to eliminate confounding variables regarding inflammatory and remodelling pathways. Inclusion criteria required that patients complete orthodontic treatment with fixed appliances using either non-extraction (no teeth were removed) or bilateral extractions (equal teeth were removed on both sides of the same arch). In addition to being in good health, patients were required to provide expressed, documented consent prior to participating in the study for the collection of both saliva and gingiva. Patients with active periodontal disease (greater probing depth or bleeding) and those who had any condition that would affect bone metabolism (such as diabetes, osteoporosis, or autoimmune disorders) were excluded from this study. The other exclusion criteria were recent usage of anti-inflammatory medications or bisphosphonates, significant smoking history, and pregnancy/lactation. These exclusions were designed to make sure that the observed biological responses were primarily due to the application of orthodontic forces rather than other systemic or environmental influences. [4]

Orthodontic Treatment Protocol

A uniform orthodontic treatment protocol was developed and applied to all study sites, providing consistent mechanical force delivery and treatment progression. All patients received fixed appliances with a 0.022 inch slot pre-adjusted edgewise system with an MBT prescription. The first phase of treatment utilized nickel-titanium archwires of increasing size for the purpose of leveling and aligning the teeth while the working phase used rectangular stainless steel archwires to produce controlled tooth movement and space closure. As such, forces used during canine retraction were standardized within the range of 150 to 200 grams in order to minimize variability of mechanical stimuli. While based on clinical response, patients were generally in orthodontic treatment for a duration of 18-24 months. This standardized approach allowed for accurate determination of biological variability as the primary predictor of treatment outcome. [5]

Clinical Data Collection Schedule

Comprehensive clinical data were collected prior to and at established intervals during the study for the purpose of correlating biomarker dynamics with clinical outcomes. At baseline, clinical data included demographic, medical, and dental history; digital intraoral scans used for three-dimensional analysis of tooth position; periodontal examination; radiographic imaging; and initial collection of samples of saliva and



gingival crevicular fluid. Longitudinal assessments of biological sampling and clinical indices (plaque and gingival scores) were performed monthly, while more detailed assessments (i.e., intraoral digital scans and radiographic imaging) were performed quarterly. At each appointment, documentation was made of the orthodontic forces being applied, as well as the degree of mobility of each tooth. Primary outcome measures were rate of tooth movement (in millimeters/month), degree of root resorption (assessed using cone beam computed tomography), total treatment duration, and post-treatment relapse (assessed during the retention phase). [6]

3. Salivary Biomarker Panel

Sample Collection and Processing

Saliva was collected using a fixed collection time window, fasted state and under strictly controlled conditions to help ensure the salivary biomarker measures will have the same pre-analytic variability and reproducibility when measured at subsequent time points or by another group of research investigators. Saliva was collected in the morning, adhering to a fixed collection time window, in an unstimulated state and used to minimize circadian fluctuations in salivary biomarker levels. In addition, saliva samples were processed by centrifuging to remove cellular material and then the supernatant was aliquoted and stored at -80°C to maintain molecular integrity of the sample. Sample quality control procedures included measuring salivary flow rate, measuring total protein concentration, and discarding samples with visible blood contamination. Implementation of these standard collection and processing procedures resulted in the generation of highly reliable and comparable analytical data for all tissues and time points throughout the study. [7]

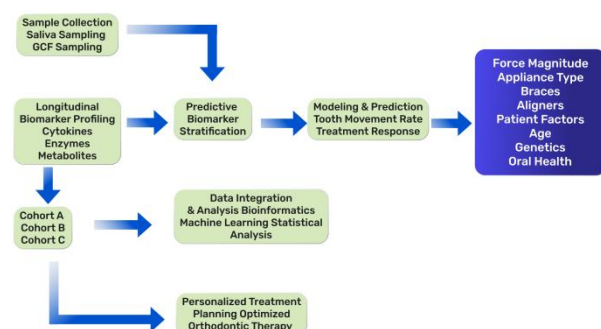


Figure 2. Multi-Cohort Analytical Pipeline for Biomarker-Based Prediction of Orthodontic Tooth Movement.

The schematic provides an overview of the longitudinal assessment of the cohort study to identify orthodontic tooth movement using salivary and gingival crevicular fluid samples for the development of personalized treatment plans based on Predictive Biomarker Stratification (highlighted in dark blue). Each branch illustrated in the schematic emanating from the salivary sampling and gingival crevicular fluid of the patients represents longitudinal biomarkers assessment and integration of the data to all patients in the study developed predictive models of tooth movement rate and treatment response. Also highlighted in the side modules illustrate the clinical integration of predictive models generated from longitudinal biomarker levels, force magnitude, type of appliance and patient-specific factors to optimize orthodontic treatment and plan for delivery of patient specific orthodontic therapy. The arrows show the correlation of biomarker level dynamics with orthodontic treatment outcomes so that the delivery of personalized orthodontic treatment can be achieved. [8]

Inflammatory Cytokine Panel

The biological response to the application of orthodontic forces initiates with inflammatory cytokines. Key cytokines that are initiated by the application of mechanical force and mediate bone remodelling processes during orthodontic tooth movement are pro-inflammatory cytokines such as interleukin- 1β , interleukin-6, tumour necrosis factor- α , interleukin-8 and interleukin-17A which are increased shortly after the application of mechanical force and support osteoclast recruitment/activation during bone resorption. These mediators demonstrate the strength of the inflammatory response and are known to significantly correlate with the speed of tooth movement while regulating the anti-inflammatory response cytokines [i.e., interleukin-10 (IL-10), interleukin-1 receptor antagonists, and transforming growth factor- $\beta 1$ (TGF- $\beta 1$)] by modulating the inflammatory response, promoting repair of tissue, and stimulating the formation of bone. The balance between the pro- and anti-inflammatory signaling pathways influences the effectiveness and safety of orthodontic treatments. Measurements of these cytokines can be performed using multiplex immunoassay techniques, allowing for the overall characterization of the inflammatory response and providing further insight into the biological response of each individual patient [9].

Bone Remodeling Markers

Bone Resorption Markers

Markers of bone resorption measure osteoclastic activity and are valuable indicators of the biological processes associated with tooth movement. Biomarkers such as C-terminal telopeptide of type I collagen and N-terminal



telopeptide represent collagen degradation products and are particularly good indicators of the degradation of bone matrix. Tartrate-resistant acid phosphatase 5b is an enzyme that is specific to osteoclasts and serves as an indication of the total number of osteoclasts and their level of activity; the receptor activator of nuclear factor- κ B ligand is essential for the regulation of osteoclast differentiation and activation. Markers of bone resorption demonstrate increased levels during the early stages of orthodontic treatment and relate to increased rates of tooth movement [10].

Bone Formation Markers

Markers of bone formation represent osteoblastic activity and the deposition of new bone on the tension side of the periodontal ligament. Procollagen type I N-terminal propeptide is used to identify collagen synthesis and the initiation of osteoblastic activity, while osteocalcin indicates late-stage formation of new bone. Bone specific alkaline phosphatase (BSAP) acts as an indicator of active mineralization processes, where osteoprotegerin (OPG) is an inhibitory regulatory factor of osteoclastogenesis through the neutralization of receptor activator of nuclear factor- κ B ligand (RANKL). The coordinated regulation of BSAP and OPG is critical for balanced bone remodeling to produce successful outcomes in orthodontics [11].

RANKL/OPG Ratio

The RANKL and OPG ratio is an important biomarker used to demonstrate the balance between bone formation and resorption. A higher ratio reflects osteoclast activity dominant over osteoblastic activity resulting in rapid tooth movement, whereas lower ratio indicates osteoblasts are predominant in activity resulting in slower tooth movement. Importantly, significant early changes in this ratio predict the kinetics of the orthodontic treatment and make it a potential biomarker for orthodontic individualized treatment planning [12].

Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs) are critical enzymes that play a large role in the degradation of extracellular matrix (ECM) components and the remodelling of the periodontal ligament during orthodontic tooth movement. Many of the MMP enzymes primarily responsible for collagen breakdown are MMP-1, MMP-8, and MMP-13; whereas MMP-2 and MMP-9 degrade gelatin and denatured ECM proteins. The activity of MMP enzymes is regulated tightly by tissue inhibitors of metalloproteinases (TIMPs). The balance of MMPs to TIMPs regulates the degree of tissue remodelling. MMPs demonstrate higher activity during active tooth movement and subsequent tissue adaptation, whereas

dysregulation of MMP activity can cause pathological results (i.e. excessive tissue degradation or root resorption). Advanced techniques for analysis, such as zymography, can provide scientists with the ability to distinguish between active and inactive forms of the same MMP enzyme, thus providing more insight into the functional processes of remodelling [13].

4. Gingival Crevicular Fluid (GCF) Biomarkers GCF Collection and Processing

Samples of GCF were taken at standardized anatomical locations to create reproducible samples and make comparisons between active teeth and the control teeth. GCF samples for this study were collected from both the maxillary canine during an orthodontic retraction and from the mandibular first molar that provided a non-moving control tooth. GCF sampling was performed at baseline and at various timepoints after the application of orthodontic retraction force (days 1, 3, 7, 14, and 28 and subsequently monthly). Using sterile paper strips (absorbant), GCF was taken from the gingival sulcus by inserting the absorbant strip for a standardized duration of thirty (30) seconds. The volume of GCF that was collected was measured and recorded using an electronic device. After measuring the volume of GCF, the sample of GCF was subsequently eluted into Phosphate Buffered Saline (PBS) and centrifuged to remove debris before being stored at -80 degrees Celsius (-112 degrees Fahrenheit) for further study. In order to obtain analytical consistencies, biomarker concentrations were purchased and normalized either by concentration of biomarker per unit volume or concentration of biomarker per unit of time with adjustments made for the total GCF volume. The purpose of this methodological standardization was to minimize variability and enable a longitudinal area to make longitudinal comparisons on the dynamics of the biomarkers. [14]

GCF Inflammatory Markers

In GCF, to date, inflammatory biomarkers have been demonstrated to increase rapidly and in an exaggerated manner immediately after the application of orthodontic force as a measure of response of local tissues to load application. The first inflammatory mediator to exhibit a significant increase in GCF and also demonstrated the greatest increase in GCF was interleukin- 1β , with the peak GCF concentration observed at days 1–3 post-force application with a concentration (10-20 times greater than baseline) faster tooth movement, as well as being associated with higher amounts of clinical inflammation, which was assessed by looking at levels of gingival inflammation. High concentrations of IL-6 were found usually to occur in the 3 to 7-day range after orthodontic force application and also correlated with root resorption,



establishing IL-6 as a biological marker of the inflammatory process and/ or one that predicts adverse effects. IL-6 had its peak amount within the first 24 hours after application of orthodontic force, showing that this period is associated with a quick biological response to orthodontic force application. After IL-6, PG E2, which is an important mediator (promoter) of osteoclast activation, peaked around day 7 post-application of orthodontic force, and the amount of PG E2 measured showed a strong correlation to canine retraction. Therefore, these biological markers are providing a progressive profile of the inflammatory response phase of orthodontic tooth movement. [15]

GCF Bone Remodeling Markers

Biological markers of bone resorption in GCF provide a greater understanding of the transition from inflammatory phase to active phase of remodeling. There was a progressive increase in RANKL over time, with peak RANKL amounts occurring between days 7 and 14, which coincides with the active differentiation of osteoclasts. The RANKL/OPG ratio at day 7 was highly correlated with the amount of tooth movement. Increased OPG concentrations were inversely related to treatment kinetics and were also higher in older individuals compared to younger individuals, thus indicating that the age differences seen in bone metabolism likely affect the OPG over time. The C-terminal telopeptide of type I collagen was first identified in the early part of the remodeling process as an indicator of root resorption related to excessive bone turnover. Collectively, this data demonstrates that GCF-derived biomarkers may help clarify the biological biological processes involved in the response of soft and hard tissue to orthodontic treatment. [16]

GCF vs. Saliva: Comparative Performance

GCF and saliva contain different inflammatory markers due to their unique biological matrixes. In the case of inflammatory cytokines, moderate correlations exist between GCF and saliva for IL1 β and TNF α . On the other hand, for biomarkers of bone remodeling (RANKL and CTx-I) the correlation was weak, reflecting the compartment-specific biological processes in both fluids. Because GCF is found in close proximity to the site of orthodontic force application and contains a higher concentration of biomarkers, it demonstrates increased sensitivity and specificity for assessment of local tissue response compared to saliva. Because saliva provides a more global view of oral inflammatory processes and is easier to obtain, it is well suited for providing routine assessments of patient compliance and to monitor improvement. Thus, the utilization of both fluid samples (GCF and saliva) may provide a more comprehensive

approach to biomarker assessment of orthodontic treatment condition. [17]

5. Clinical Predictors of Tooth Movement Rate Demographic and Clinical Variables

The clinical predictors for an orthodontic tooth movement rate will be determined to have both a demographic impact as well as a clinical effect. Age is the most critical factor in estimating these levels. Results show that younger adolescents generally have the highest movement of canines, then younger adults, followed by older adults as bone density decreases with age. Differences in sex are also apparent, as females show somewhat faster movement than males. The role of gingival health or periodontal status also played a significant role in the speed of tooth movement because the patients with mild inflammation of the gingiva had a higher rate of movement than patients who had healthy gingiva and this suggests that low-levels of inflammation are what stimulate osteoclast activity. In addition, the shape of a tooth root can also play a role in how quickly or slowly a tooth moves because teeth with conical roots tend to be able to be moved more expeditiously than those with complex root shapes do due to the amount of periodontal ligament surface area available for tooth movement. [18]

Force Magnitude and Duration

The magnitude and duration or stability of orthodontic forces applied during tooth movement are two of the major predictors of biological responses. Teeth that were moved with optimal orthodontic forces between 150 - 200 grams will present themselves as having both efficient tooth movement and biological responses to the force applied. There is also a difference in force application depending on the force delivery system type that will impact the biological response, as elastomeric chains showed significant loss of force overtime and coil springs tend to provide consistent force over time; therefore, coil springs allow for more predictable tooth movement patterns. An increased number of prior orthodontic force studies have documented that higher magnitude orthodontic forces will also lead to an increase in the expression of inflammatory mediators such as interleukin-1 β and prostaglandin E2 at the site of the tooth, however, applying excessive orthodontic forces greater than what a tooth can tolerate will create a relative transient ischemia of the periodontal ligament; therefore, due to the compromised ability of the periodontal ligament to support tooth movement due to the loss of blood supply, excessive orthodontic forces will reduce the efficiency ofThe value of appropriate levels of force, rather than excessive force, is important



because it allows for optimal biological response as well as minimizing negative biological responses. [19]

Tooth Movement Rate Trajectories

Distinct biological responses have been found for different groups of patients, with three main groupings of patients established; rapid movers, moderate movers, and slow movers. Each of these patient groupings exhibits a unique clinical and biological profile, with rapid movers generally younger with higher baseline inflammatory levels, and slow movers usually older with lower inflammatory base levels and lower biological responses. These findings highlight the necessity for individualized treatment plans as well as predictability with the use of biological markers. [20]

6. Biomarker Dynamics During Orthodontic Tooth Movement

Early Phase (Days 0–7): Acute Inflammatory Response

In the early phase of orthodontic treatment, there is a rapid inflammatory response following the application of force characterized by a rapid increase in both salivary and GCF biomarkers. A number of pro-inflammatory cytokines, including interleukin-1 β , interleukin-6, and tumor necrosis factor- α , will have an increase in concentrations that will peak in the first couple days after force is applied, with the greatest concentrations seen in GCF as compared to saliva; however, Prostaglandin E2 and RANKL will also continue to have increases during the early phase of tooth movement. The increase of prostaglandin E2 and RANKL is important to osteoclast activation; therefore, the initial interleukin-1 β response has been shown to be predictive of later rates of tooth movement. [21]

Mid-Phase (Days 7–28): Bone Remodeling Phase

During the mid-phase of tooth movement, active bone remodeling is taking place with generally a sustained increase in RANKL, increased RANKL/OPG ratio, and increased bone resorption markers such as CTX-I and TRAP5b. The ability to monitor ongoing osteoclast activity and monitor tooth movement rates, reflects the relative level of RANKL (receptor activator of nuclear factor κ B ligand) to OPG (osteoprotegerin) in the relationship of these two factors gives indication about the extent of tooth movement during this initial phase of treatment. A higher RANKL/OPG ratio during this period results in faster rates of tooth movement ($p=0.00025$) demonstrating the relevance of this ratio as a critical biomarker for determining the extent and expected biological activity related to remodeling of tooth structure. [22]

Late Phase (Days 28+): Adaptation and Stability

During the late phase of treatment, inflammatory markers are gradually returning to their baseline level, representing the resolution of acute inflammation. However, in patients who are currently undergoing active closure of orthodontically-created spaces, there may be elevated levels of bone remodeling markers present, which indicates that continuing biological activity exists within the body. The markers for cumulative measures of inflammatory burden are found in saliva and generally reveal a slower rate of normalization compared to GCF; however, GCF contains localized tissue dynamics that possess a higher degree of sensitivity compared to salivary measures. [23]

Inter-Individual Variability

Significant inter-individual variability exists in the levels of the identified biomarkers for all patients at baseline, indicating a broad range of biological responses to mechanical loading or applied orthodontic forces presents. High coefficients of variation (CV) exist for the primary biomarkers of interleukin-1 β and RANKL/OPG ratio and demonstrate marked variability amongst all patients. These findings demonstrate that absolute values of the biomarkers may be less clinically significant than the relative change over time in an individual patient. As a result, longitudinal measurements and individualized baseline comparisons are critical to effective dental/orthodontic treatment using biomarkers. [24]

7. Biomarker Predictors of Tooth Movement Rate

Univariate Analysis

Univariate analysis of the combined data derived from GCF and saliva from each patient demonstrated that both GCF and saliva biomarkers are strongly correlated to the speed of orthodontic tooth movement; therefore, GCF and saliva markers are both valid as early biological indicators of treatment kinetics. Patients with baseline interleukin-1 β levels greater than five pg/mL exhibited a significantly higher mean movement rate than patients with lower levels of interleukin-1 β . This may suggest that a pre-existing pro-inflammatory condition can "prime" the periodontal ligament and the surrounding bone for increased remodeling. In addition, patients with a baseline RANKL/OPG ratio greater than one had increased rates of tooth movement. This indicates that an environment conducive to osteoclastogenesis and net bone resorption exists in these patients prior to the application of an active force. [25]

The predictive utility of salivary biomarkers increased after initiation of treatment. Salivary interleukin-1 β levels greater than 10 pg/mL at seven days post-treatment were associated with a significantly faster rate



of canine retraction. This indicates that the early inflammatory response is an important factor in determining the rate of tooth movement. Additionally, elevated levels of salivary CTX-I at the same time point predicted an increased amount of tooth movement, indicating that the biological processes associated with collagen degradation and the resorption of bone were already established in faster responders at that time point. These data lend support to the hypothesis that biomarker changes occurring soon after the application of a force provide greater predictive value for subsequent tooth movement than baseline biomarker values. [26]

Gingival crevicular fluid biomarkers may be even better able to distinguish between treatments than salivary biomarkers because they are located closer to the site of force application, and because of the relatively greater concentration of these markers at the site of force application compared to their concentration in saliva. Day 3 Results: Patients that had GCF interleukin-1 β levels greater than 20 pg/ μ L demonstrated significantly faster tooth movement than patients who had interleukin-1 β levels below 20 pg/ μ L. Findings underscore the acute local inflammatory response in the periodontal environment. Further, elevated GCF prostaglandin E2 levels were also significantly associated with increased rate of tooth movement, corroborating the established role of prostaglandins in promoting the differentiation and activation of osteoclasts. Collectively, univariate analyses found that both systemic inflammatory markers and site-specific inflammatory markers can provide clinically relevant information regarding movement kinetics; however, GCF biomarkers may provide greater sensitivity in detecting local biological responsiveness. [27]

Multivariate Model

Although many aspects reported in the univariate results appear valid and of biological significance, a more clinically useful/valid interpretation of candidate biomarkers would involve identifying independent predictors of rapid orthodontic tooth movement. In their adjusted multivariable model the authors noted that being under 20 years of age was associated with acceleratory movement rate, reflecting our understanding of the bone turnover and stage of development, role in orthodontic treatment success. High GCF interleukin-1 β levels at baseline independently predict rapid movement, suggesting those patients initiating treatment with the greatest local inflammatory response will predictably move faster extrinsically. At day 7, a higher salivary RANKL/OPG ratio remained a significant predictor which may reflect a sufficient clinical signal to facilitate prediction of speed of movement beyond localised

inflammation. As expected, the variable, peak GCF prostaglandin E2, also contributed to the predictor model independent of the other biomarkers identified earlier. [28] As with many areas of predictive modelling, this is a strong model (it achieves AUC= 0.85). Sensitivity and specificity are strong enough to consider potential clinical translatability when identifying patients similar to Kristy who would be expected to undergo accelerated tooth displacement. Thus, the rate of tooth movement should not be conceived solely as having a central predetermined 'Waldo' - the bioavailability of factors regulates tooth movement rates in concert with patient ages and local levels of inflammation. Practically, the model insinuates a biomarker-guided strategy in which clinicians will expect the Kristys to move more rapidly or the Marcias, Marinas and Peters more slowly and adjust archwire mechanics, appointment waiting times or risk tolerance accordingly. The overall performance of the model was strong, with an area under the curve of 0.85, demonstrating good discrimination between rapid and non-rapid movers. Sensitivity and specificity were both high enough to support potential clinical translation, particularly for identifying patients likely to exhibit accelerated tooth displacement. Importantly, this model illustrates that the rate of tooth movement is not determined by a single biomarker, but rather by the interaction of patient age, local inflammatory intensity, and the balance of bone remodeling mediators. From a practical perspective, the model supports a biomarker-guided approach in which clinicians may anticipate faster or slower movement early in treatment and adjust mechanics, appointment intervals, or risk monitoring accordingly. [29]

Predictors of Slow Tooth Movement

Analysis of predictors of slow tooth movement yielded a separate biological signature corresponding to lower inflammatory activation and a more anti-resorptive balance between bone-remodellers. Determined 2 was a value older than 30 years. This prediction resonates with the orthodontal observations relating to reduced cellular turnover and decreasing skeletal adaptability accompanying age. Days one and four were subjects that move slowly at eight weeks of 168 μ g/l. This suggests that local gene expression of osteoprotegerin - a 'decoy', RANKL-blocking molecule' - positively promotes a biological environment in which RANKL-mediated osteoclast activation is inhibited. Such observation is important as it relates to the biological plausibility of orthodontic forces transducing poorly to biological remodelling. The multivariate predictability of older patients helps guide expectations of treatment duration; meaning, presumably, that Marcias's orthodontics could be extended duration of the appurtenant age alone. [30]



Low RANKL/OPG ratio at day 14 was perhaps the strongest predictor of a slow mover, suggesting that lack of a sufficient shift to an osteoclast-rich state during the remodeling phase is restricting tooth movement. Likewise, if a patient had a low peak GCF interleukin-1 β response they were more likely to be in the slow-mover category, suggesting that not activating sufficient early inflammation may lessen downstream bone remodeling. This provides further credence from the biological standpoint that slow movement may be characterized by decreased inflammatory responsiveness and a lack of osteoclast recruitment. Clinically, recognition of this phenotype may warrant longer treatment timelines, more conservative force protocols, and more frequent reassessments of mechanical effectiveness. [31]

8. Biomarker Predictors of Root Resorption Incidence and Severity of Root Resorption

Root resorption was one of the most serious adverse effects in clinical outcomes at the completion of treatment, with almost half of patients exhibiting some level of instrumented root structure loss as measured on cone beam computed tomography. In the majority of patients this was mild but in a clinically significant minority the loss of root structure was more severe. The most vulnerable teeth were the upper central incisor teeth and upper canine teeth, consistent with their known exposure to high forces over extended periods during full arch movements of teeth. In summary, root resorption is not an uncommon finding in orthodontic treatment and warrants attempts to identify high risk patients in advance of formal evaluation of clinical outcome post appliance treatment by identifying the biological individuals in whom such loss of structure is evident before it becomes manifest. [32].

Biomarker Predictors of Root Resorption

Biomarkers assessed prior and on treatment were associated with risk of resorption suggesting that this susceptibility arises from the biological endowment of the patient but also relates to the intensity of the biological response evoked in the tissues by the orthodontic forces applied. GCF interleukin-1 β levels in baseline GCF were proportional to the risk of resorption in orthodontic patients, which suggest that pronounced local inflammation may not only accelerate the speed of tooth movement, but indeed lead to the breakdown of underlying tissue around the tooth in excessive amounts. Similarly baseline, reduced levels of salivary osteoprotegerin were associated with higher risk, corroborating the importance of both local and systemic signs of remodeling on the structural outcome of treatment. [33]

During treatment, the levels of CTX-I in GCF at day 14 after tooth movement was strongly linked to the severity of the resorption indicating that collagen degradation within the local environment ofOverall, a high GCF RANKL/OPG ratio at day 14 also predicted increased resorption risk, once again emphasizing the role of excessive osteoclastogenic signaling in this complication. Combined into a multibiomarker model with GCF interleukin-1 β and CTX-I, the predictive performance was strong (AUC = 0.82). "Our findings pave the way for future studies directing biomarker monitoring strategies toward early identification of those at risk for pathologic root resorption, which may provide opportunities for risk-adapted treatment strategies to reduce resorption, while retaining the efficiency of overall therapy. [34]

Mechanistic Insights

The causes of the biomarker-associated root resorption are hypothesized to arise through excessive, poorly controlled osteoclast activity. The high RANKL/OPG ratio suggests a strong signal for osteoclast differentiation and resorption, capable of persisting beyond simply local alveolar bone, to root dentin and cementum as well. CTX-I is a breakdown marker of collagen, and given root dentin is composed of type I collagen, high levels may signify not only physiological resorption of bone, but also pathologic degradation of roots. The persistence of elevated pro-inflammatory cytokines such as interleukin-1 β and tumor necrosis factor- α beyond the very earliest portions of treatment may also signal chronic inflammation that may maintain catabolic signaling through active degradation. Concurrently, levels of appropriate anti-inflammatory mediators such as interleukin-10 and transforming growth factor- β 1 may be insufficient to properly switch off osteoclasts. The frame of reference of root resorption as overzealous in which the balance of necessary remodeling, and protective resolution of remodeling has failed. [35]

9. Biomarker Predictors of Treatment Duration and Relapse

Treatment Duration

Biomarker Predictors of the Duration of Treatment and RelapseLength of TreatmentThe actual time period of treatment for each patient was titrated by the biological tempo of tooth movement itself, with it being noted that slow movers would need a much longer treatment length than quick movers.This concept is intuitively appealing from a mechanical perspective, but the biomarker data shed light on which underlying biological mechanisms are involved. Those with high osteoprotegerin at baseline tended to have longer hemorrhoid therapy (again pointing to an anti-resorptive biological profile slowing



orthodontic displacement). A low peak interleukin-1 β response also related to longer hemorrhoid therapy (insufficient early inflammatory activation delayed the advent of effective remodeling). In summary, early assessment using available biomarkers is useful in anticipating duration of treatment better than clinical planning alone. [36]

Relapse at 12-Month Retention

Relapse during the 12 month retention was related to biomarker profiles of those at high risk of relapse. Those with relatively high GCF interleukin-1 β at day 7 began their retention period at much higher risk of relapse. Additionally, those who had a high GCF RANKL/OPG ratio at day 14 were also more likely to relapse. Interestingly, continued elevation at interleukin-1 β at month 12 after active treatment completion was predictive of relapse indicating that a biological “too hot to handle” may continue to destabilize this periodontium and the supporting bone. [37]

The suggested mechanism is persistent low-grade inflammation that may continue to promote bone remodeling and jeopardize having arrived at a stable tissue state post-treatment. In such subjects, even after apparent clinical “completion” of treatment, net remodeling may still be active and the periodontal ligament and surrounding bone (more prone to displacement) are biologically “younger.” This raises the interesting possibility of developing a biomarker-informed retention protocol where patients with a continuing inflammatory signature would be treated with longer or more intensive retention protocols. [38]

10. Inter-Cohort Comparisons

Adolescent vs. Adult Cohort

Comparisons between adolescents vs adults demonstrated fundamental biological differences in response to orthodontics that are reflected in biomarker trends. Adolescents demonstrated substantially greater inflammatory and remodeling responses as shown with markedly elevated starting levels of interleukin-1 β and a much steeper peak in the RANKL/OPG ratio in the early stages of treatment in adolescents. This greater biological responsiveness translates into faster rates of tooth movement, and adolescent patients display a significantly greater amount of tooth movement per month during treatment than adults. Conversely, adult patients have a greater baseline level of osteoprotegerin (an anti-resorptive signal), and osteoprotegerin levels decline more slowly than kyoto. This might illustrate a prolonged remodeling activation that is less profound than the adolescent response. [39]

Clinically, this tended to track with a faster rate of orthodontic movement and less root resorption in adolescents. Adults, however, experience a greater degree of root resorption overall, and more slowly progress through treatment, presumably on account of slower cellular turn-over, differences in vascularity and reduced regenerative capacity of periodontal tissues. For treatment implications, adolescents may be more amenable to treatment with lower magnitudes of force that might compromise less, while allowing for more efficient movement. Adult patients may well take longer to go through treatment, and may benefit from adjunctive procedures which attempt to increase the rate of remodeling and decrease treatment time. [40]

Healthy vs. Periodontal Risk Cohort

With respect to patients with healthy periodontal status compared with those with prior periodontal risk factors, a phenotype driven by elevated biomarker status was present. Those with periodontal risk status had markedly higher levels of interleukin-1beta, tumour necrosis factor-alpha and matrix metalloproteinase -8, which are suggestive of a pre-activated state in terms of the inflammatory milieu seen in the periodontal tissues. This “primed” state equated to a modest but significantly increased (7.8%) rate of tooth movement, with effects attributed to preload osteoclast activation and more efficient orthodontic displacement of teeth. [31]

However, with this comes a far greater incidence and severity of root resorption, which is no doubt mediated by this increased proteolytic and inflammatory activity in terms of accelerated breakdown of mineralised tissue such as root dentin - all of which has clear clinical ramifications. This speaks to the need for careful selection of patients, and optimising patients well prior to orthodontics. Periodontal therapy performed prior to orthodontics is key to reducing baseline inflammatory loads, not to mention the need in these patients for close attention in terms of radiographic and biomarker monitoring throughout therapy for detecting early signs of pathological resorption. [32]

Sex-Based Differences

When comparing Sex-based differences, whereby there were relatively minor yet biologically important discrepancies in orthodontic response, primarily through differences in inflammatory signalling. For females, wherein slightly accelerated tooth movement results occurred, greater baseline levels of interleukin-1 beta and prostaglandin E2 were noted when compared to males, perhaps reflected by alterations in hormonal status (oestrogen). This also suggests that female patients may be generally more responsive in orthodontic treatment,



though this does not appear to lead to greater occurrences of resorption risk levels or severity, thus are hence inconsequential in treatment expectations. [43]

Regardless of these differences in kinematics, the females did not exhibit a higher incidence or severity of root resorption, indicating that while hormones may accelerate the biological process of remodeling, they do not favor the development of adverse structural consequences. Thus, minor sex-based modifications to orthodontic therapy are unnecessary, although in the awareness of accelerated biological responsiveness in the female patient, certain clinical wisdom of treatment phase takeover would be instructed. [34]

11. Clinical Translation and Implementation

Proposed Clinical Algorithm

The incorporation of salivary and gingival crevicular fluid biomarkers into clinical orthodontics paves the way for a structured biomarker-derived decision-making algorithm. At baseline, simultaneous screening using interleukin-1 β , RANKL/OPG ratio and CTX-I allows the classification of patients into biologically distinct rapid or slow movers. This initial stratification supports treatment planning by helping the clinician still pre-force application "know thy patient" regarding treatment kinetics and risks. [35]

For these patients, their rapid movers can be treated using the same force magnitudes as previously planned, with vigilant monitoring of root resorption, knowing that, biologically, the movement should be rapid. In contrast, additional adjunctive strategies could help the slow movers to remodel better. Early, post-initiation of treatment monitoring (Days 7-14) represents another critical gate where clinicians can use biomarker screening to recognize "okay, the biology is behaving as it should", or "no, it's not" where the physics of teeth moving normally may need to be dynamically reset via the magnitude of the force used (or some adjunctive therapy). Long-term "gating" of treatment success might occur internally via periodic salivary and/or monitoring of radiographic evidence of successful treatment to avoid failure while helping the practitioner, dynamically adapt treatment strategies. [36]

Adjunctive Therapies to Accelerate Movement

Adjunctive therapies designed to accelerate orthodontic tooth movement act through modulation of the inflammatory/remodeling pathways uncovered through biomarker exploration. Micro-osteoperforations may be the least effective here, effecting a local inflammatory response leading to a local increase in IL-1 β and RANKL/OPG ratio in the affected area, accelerating the

osteoclast so that tooth movement is accelerated. Alternatively, low-level laser therapy here should achieve many, safer positions, meanwhile "talking" the inflammatory system down so the procedure backs off biologically. Although low-level laser therapy also subtly aids tooth movements in some studies, the variation and differences in movement rates across studies displace laser use into a "caveat emptor" category for many orthodontists using laser therapy. [37]

Nutritional factors, particularly vitamin D status, also play a role in bone metabolism. Correction of vitamin D deficiency may normalize the RANKL/OPG balance and improve the efficiency of remodeling in slow responders. Experimental approaches using local delivery of prostaglandin E2 or RANKL show promise in preclinical studies, but are not yet approved for routine clinical use. Together these strategies highlight the potential for biomarker-guided adjunctive therapy to optimize treatment outcomes. [38]

Risk Stratification for Root Resorption

Biomarker-based risk stratification enables identification of patients at increased risk for root resorption using a high-risk signature. High-risk patients have elevated baseline GCF interleukin-1 β , increased CTX-I during treatment, and a high RANKL/OPG ratio at the onset of treatment, indicating excessive osteoclast activity and tissue breakdown. In these patients, treatment protocols must be modified to minimize harm by applying less force, increasing the frequency of radiographic monitoring, temporarily discontinuing active forces for tissue recovery if needed. [39]

This proactive over reactive management approach allows early identification of risk by biomarker monitoring. It also supports treatment efficiency and biological safety in efforts to ensure optimal tooth movement outcomes without causing structural damage. [30]

Cost-Effectiveness Considerations

Biomarker-guided orthodontic care also shows potential for cost saving from a health economics perspective despite the cost of testing. Cost of combined salivary and GCF biomarker panel is modest compared to standard laboratory tests. Despite the headache of further biomarker tests every few months, the downstream dividend is shorter treatment time, less complications, reduced need for corrective maneuvers, and happy patients. [31]



A shortened treatment time by several months can lead to serious cost reduction to the patient and the medical community while root resorption can be detected early enough to prevent nasty complications which are a real costly nuisance; lower value treaties with the convenience of self-space close efficiency. [32]

12. Future Directions

Point-of-Care Biomarker Devices

A broader presence of point-of-care technology arrives at the feet of the new biomarker technologies in true clinical practice. “Rapid saliva assays utilizing lateral flow technology are able to detect biomarkers for mediators of tooth movement such as interleukin-1 β and RANKL.” Immediate salivary biomarker assays with these developers may make offsite laboratory determination a thing of the past. Apparently, these items are closer to arriving on the shelf than chrome molar bands. Providers can measure the potential inflammatory status of teeth in seconds and proceed to clinical action. Gingival crevicular fluid collection devices with built-in biosensors may allow chair-side detection of mediators of inflammation; sensitivity and specificity should not be different from that of laboratory methods. [43]

Smart orthodontic appliances with embedded sensors that could monitor forces during application as well as biological changes with use could also yield fascinating data on the mechanics of orthodontia. The delivery of orthodontics may be on the cusp of a data revolution. [34]

Genetic and Epigenetic Markers

Genetic Markers and Epigenetic Markers Genetic and epigenetic profiles can take personalisation a step further by matching patient susceptibility for specific biological events. Lastly, Single nucleotide polymorphisms in genes influencing inflammation and bone remodeling (interleukin-1 β , RANKL, osteoprotegerin) correlates with tooth movement rate and risk for root resorption. Also, DNA methylation patterns within inflammatory gene promoters may shape individual responsiveness to orthodontic loads [45].

These, as well as prior markers, may be bundled into polygenic risk scores that guide predictions of treatment outcome prior to initiation. While usefulness falls short currently, ongoing research will likely enhance predictive performance [46].

Proteomic and Metabolomic Signatures

Next, omics—proteomic and metabolomic signatures— not limited to common inflammatory markers and bone turn over indicators. Instead discover new “halls of

mirrors”, more sensitive indicators for tooth movement involving complex biophysical interactions. Salivary proteomics, perhaps even using RNA exosomes, will reveal previously unmeasured inflammatory and remodeling factors. Metabolomic profiling proves through metabolite panels nitric oxide production and lipid mediators reflect metabolism, potential underload or overload points [47].

Although currently limited in clinical realization, the ultimate result is more biomarker panels with better predictive performance approaches. Analytic advancements rendered readily accessible lead to rapid integration into clinical assessments [50].

Personalized Orthodontic Protocols

The end game of biomarker integration is the personalized orthodontic protocol. Instead of “one-size-fits all” or “the standard of care”, a biomarker guided selection of forces being applied exploits patient responsiveness for maximum efficiency with minimal risk. Likewise, tailoring the time between appointments according to movement speed and metabolic activity can lead to better quality treatment and greater patient satisfaction. [49]

Retention strategies can also be personalised, with long-term retention advised for patients at increased risk of loss of correction based on inflammatory markers. The inclusion of biomarkers reflects a step towards ‘precision’ orthodontics - treatment individually targeted to the biology of each patient rather than adhering to ‘one-size-fits-all’ protocols. [50-51]

13. Conclusions

Summary of Key Findings

Orthodontic tooth movement is mediated through a cascade of biological events which can be reliably measured by examining salivary and gingival crevicular fluid biomarkers. Each phase of treatment is associated with characteristic biomarker profiles involving early inflammatory peaks and subsequent activity of bone remodeling. In essence, elevated interleukin-1 β , a raised RANKL/OPG ratio, and a relatively younger age positively predict an increased rate of movement, whereas risk of root resorption positively correlates with a heightened inflammatory activity and excessive root resorption.

Inter-cohort comparisons show that age dictates continued bone matrix turnover and extrinsic environmental factors, such as periodontal status, influence the response to orthodontic force. Models



using biomarkers can predict orthodontic tooth movement with good reliability, and thus have tremendous potential to be included in a clinician's toolset.

Clinical Implications

Translating biomarker applications into orthodontic realms entails the dentist effecting a change towards a future where predicting treatment dynamics can be realised, allowing treatment to be individualised with optimisation of forces employed for movement of teeth, and prediction of potential adverse treatment effects. Consequently, improving clinical outcomes, enhancing patient satisfaction, and supporting more efficient use of health resources.

Research Priorities

Further studies should aim to explore, on a large scale, what constitutes significant deviation from the parameters defined and strengthen protocols towards rapid point-of-care assay format. Prioritising genetic and multi-omic data to integrate into osteoporosis research. Interventional studies comparing biomarker-guided care against current guidelines in delivering clinical benefit to reinforce translation efforts into standard practice.

The Road Ahead

The confluence of biomarker science with digital technologies and AI promise precision orthodontics within reach, where non-invasive monitoring, real-time decision support, and personalised treatment protocols mean orthodontic care will be more predictive, preventive, and appropriate worldwide.

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