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Research Article

BIOMARKERS IN ENDODONTICS – A LITERATURE REVIEW

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Abstract

Biomarkers are functional elements at the a cellular or molecular level, playing an important role in the health and disease. These biomarkers can aid in the appropriate diagnosis of the pulpal and periapical condition and thereby aid in choosing the right treatment plan. This review aims at providing a brief information of few biomarkers of specific pulpal and periapical condition and their role.

Keywords: Biomarkers, Periapical condition, Pulpal disease, PERIAPICAL Periodontitis

INTRODUCTION

A biomarker is a measurable entity that reflects normal biological processes, disease progression, or the body's response to a therapeutic treatment. The term is often used to describe any molecule or substance found in a biological fluid that is associated with a specific condition, its progression, or its diagnosis. National Institutes of Health Biomarkers has defined the description of a biomarker as a "characteristic that is objectively measured and evaluated as an index of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (1).Essentially, a biomarker can be seen as an indicator of molecular interactions at the functional, psychological, biochemical, or cellular level. Biomarkers are being widely used in research as well as in the medical field to assist in diagnosis and treatment. This review aims at providing a brief information about certain specific biomarkers involved in pulpal and periapical conditions and their respective role.

Importance of Biomarkers in Endodontics

Making accurate diagnostic decision in the clinical management of deep carious lesions and the diagnosis of pulpitis is a highly complex. The decision of whether to perform vital pulp therapy or root canal treatment is crucial, as it largely depends on the clinician's ability to differentiate between the various stages of pulpal inflammation and make an accurate diagnosis. For diagnosing endodontic conditions, clinical signs &symptoms are considered rather than histologic findings, since histologic pulp tissue examination is not possible in most of these situations. Current diagnostic method aims at determining the state of pulpal inflammation based on patient's symptomatic history, pulpal sensibility tests and radiographic evaluation. However, none of these methods accurately diagnose the true histological status of the pulp. Molecular-based diagnosis has gained significant attention, particularly in identifying the inflammatory molecules involved in the inflammatory response within the pulp and periradicular tissues following bacterial infection.

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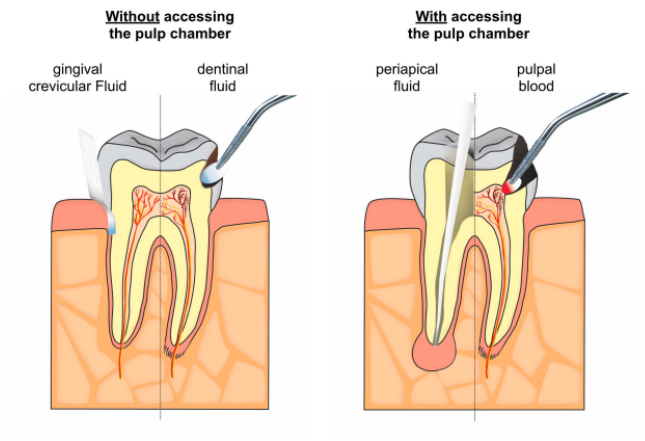
Identification of these molecular markers / biomarkers can thereby play an important role in understanding the pathogenesis of dental caries, pulpal and periapical pathosis and thus help to diagnose them accurately. This would guide the clinician regarding the appropriate clinical treatment. Pulpal inflammation involves several biological processes evaluable at the macroscopic, microscopic, and molecular levels. A range of biological molecules, including proteases, growth factors, chemokines, and cytokines, are released in response to various pulpal and periradicular conditions. The quality and quantity of these mediators are crucial in determining the course of inflammation, particularly in relation to the type of immune response generated in the tissue. Some mediators guide and amplify the process of inflammation (eg: interferon-g, interleukin [IL]-2, IL-12, tumor necrosis factor [TNF]-a). While other mediators are responsible for tissue repair (eg: IL-10, IL-4). Additionally, the carious lesion model describes that deeper lesions contain higher levels of inflammatory mediators and exhibit increased pulpal inflammation. As a result, these mediators may serve as biological markers, providing a more precise and biologically reliable method for diagnosing the inflammatory condition of the pulp in endodontics.

Methods of obtaining the sample

Measurable levels of molecules can be detected not only in pulp tissue but also in pulpal blood, dentinal fluid, and gingival crevicular fluid all of which can be non-invasively collected and analyzed without the need to extract pulp tissue. Samples / analysts for analysis of various biomarkers can be done via different approaches both from the pulp directly as well as indirectly from tissue fluid (fig: 1).

Direct Method	Indirect Method
Pulpal Blood	Dentinal Fluid
Periapical Fluid	Gingival Crevicular Fluid / GCF
	Saliva



Pulpal Blood

Blood and its cellular components are a crucial of specific and the non-specific immune system. Pulp blood may contain factors distinct from those in peripheral blood, suggesting that this fluid provides valuable site-specific information. Dr. Florian Prader from the University of Zürich was the first to study a pulpal blood hemogram. While pulpal blood and whole pulp tissue offer a direct insight into the intrapulpal condition, obtaining them requires accessing the pulp chamber and tissue, making the method more invasive. It has been found that invasive methods of entering the pulp space reduce the pulp survival chance.

Periapical Fluid

The term "periapical fluid" / "periapical tissue fluid" refers to the extracellular transudate fluid present in the periapical area of the tooth. In conditions like asymptomatic apical periodontitis, the periapical fluid acts as an inflammatory exude, which gradually gets accumulated with various Pro and anti-inflammatory signaling molecules. These molecules play a vital role in coordinating the cellular responses that result in local tissue damage and the onset of clinical symptoms.Recently, periapical tissue fluid has been analyzed at both the proteomic and transcriptomic levels by clinically collecting it from root canals using paper points.Several studies have shown that these fluids can be harvested for local biomarker molecular analysis, providing proof-of-concept for their potential in diagnostic, prognostic, and predictive applications. But the major disadvantage of this method is the need to extirpate the pulp (i.e; pulpectomy) to obtain the sample.

Dentinal Fluid

Dentin fluid is an extracellular fluid that is present within the dentinal tubules and it contains inflammatory mediators and vasoactive compounds which are linked with inflammation. It also represents as a noninvasive source of obtaining inflammatory markers. Dentinal fluid may serve as a surrogate source of biomarkers with diagnostic potential, reflecting the state of the pulp and helping to differentiate between symptomatic irreversible and reversible pulpitis. The idea of sampling dentinal fluid from the dentin wound to assess the condition of the pulp originated with Professor Pashley's research group. Although the initial evidence indicated that these mediators can be assessed, challenges remain with the protein yield, as well as the need to remove any existing restoration or, in certain cases, an initial cavity into the dentin to gain access to the fluid, making it invasive.

Gingival Crevicular Fluid / GCF

Gingival crevicular fluid (GCF) is a plasma-derived exudate that can be collected non-invasively from the gingival margin or within the gingival sulcus. It holds considerable diagnostic potential as a source of biomarkers for periodontal disease at the marginal level. GCF analyses may be promising due to the ease of application. Making use of these biomarkers to assess marginal periodontal diseases is a widely accepted concept. A key limitation of using GCF biomarkers is that tissue inflammation, regardless of its underlying cause, is a non-specific response of the innate immune system, which complicates the ability to differentiate molecularly between marginal and apical periodontal inflammation.

Saliva

Saliva is a versatile biological fluid containing variety of biomarkers mirroring both physiologic and pathophysiologic states of the oral & systemic health. Saliva is the most opted medium to collect the biomarkers non-invasively from the oral cavity, due to its ease at sampling and the wide range of microbiological and immunological biomarkers it can provide for analysis. Salivary sampling can be used to detect diagnostic and prognostic biomarkers for both local and systemic inflammation.

Different Biomarkers and their use in Endodontics

Inflamed pulp is seen in an increasing number of inflammatory cells with synthesis of pro-inflammatory mediators, such as proteases, growth factors, chemokines and cytokines, all of which exacerbate the immune inflammatory response (2,3).

Types of biomarkers that have been described and could be used in Endodontic research (4):

Information	Systemic Local
Diagnostic	Detect disease, its recurrence, or progression
Prognostic	Provide insight into the natural history of disease (recurrence, survival)
Predictive	Predict response to treatment

Different types of the biomarkers can be easily isolated from various biological samples like saliva, blood, and dental tissues (5):

1. **Inflammatory Biomarkers**: In response to the endodontic infection, various inflammatory biomarkers are released, such as : IL 1, IL 6, IL 8, TNF Alpha, Prostaglandins (PGE2) etc
2. **Tissue destruction biomarkers**: These indicate the breakdown of pulpal and periapical tissues, which can occur in response to chronic inflammation or infection within the pulpal tissue or periapical tissue. Such as: MMPs, Deoxypyridinoline / DPD
3. **Microbial Biomarkers**: These biomarkers are directly associated with the presence of pathogenic microbes in endodontic infections, including bacterial DNA or RNA and endotoxins released by the bacteria.

4. **Regenerative Biomarkers**: Indicative of tissue healing and regeneration, critical in scenarios which focuses on tissue repair (VPT, REP etc)

The quality and quantity of these mediators play a vital role in shaping the progression of inflammation, especially in terms of the type of immune response triggered in the tissue.

Table 1. Biomarkers of diseases in pulpal and periapical tissues (6)

DISEASE	BIOMARKER
Reversible Pulpitis	1. Prostaglandin E2 2. Interleukin 1Beta 3. Osteocalcin 4. Inducible Nitric 5. Oxide synthase (iNOS) 6. CXC Chemokine 7. ligand 10 (CXCLC 10) 8. Human b defensins (hBDs)
Irreversible Pulpitis	1. Substance P 2. MMP – 3 3. IL – 8 4. TNF alpha 5. Manganese superoxide dismutases 6. Prostaglandin F2 alpha
Asymptomatic Apical Periodontitis	1. RANKL & OPG 2. MMP-2 3. MMP - 9
Symptomatic Apical Periodontitis	1. Tartrate Resistant Acid Phosphatase (TRAP or TRAPase)
Periapical Abscess	1. Mesenchymal Stem cell Marker
Periapical Cysts	1. IL-6
Root Resorption	1. Dentin phosphoprotein (DPP) 2. Dentin Sialoprotein 3. Dentin Sialophosphoproetin 4. OPG/RANKL 5. microRNA
Periapical Granuloma	1. FoxP3

Matrix Metalloproteinases

MMPs are synthesized by cells of connective tissue such as fibroblasts, osteoblasts, and odontoblasts and secreted into the extracellular matrix.They play an important role in dentin matrix formation during Dentinogenesis, modulating progression of dental caries as well as secondary dentin deposition. MMPs also shown to be a participant in the process of reversible pulpitis, irreversible pulpitis and periapical inflammatory pathosis. It is believed that matrix metalloproteinases (MMPs), such as MMP-2, MMP-8, and MMP-9, play a specific role in various dental pathologies.
Current literature indicates that pathological pulp tissue in permanent teeth exhibits significantly higher levels of MMP-1 and MMP-2 compared to healthy pulp tissue (7). Although they tend to have low expression and activity in adult tissues, but their production shoots up at the onset of any destructive pathologic process. Various matrix metalloproteinases (MMPs) have been identified in the dentin-pulp complex through the use of polymerase chain reaction (PCR) and immunohistochemistry techniques.

Matrix Metalloproteinase – 3 (MMP-3)

MMP – 3 has said to have a unique role in pulpitis that other MMPs do not share. The concentration of MMP – 3 is found to be higher in reversible pulpitis than in irreversible pulpitis (8). Studies show that MMP 3 produced during reversible pulpitis brings about degradation of surrounding collagen, leading to changes in the structure of the Extracellular matrix, inflammation, promoting angiogenesis and accelerates pulpal heling through reparative dentin formation (9).

Matrix Metalloproteinase-8 (MMP-8 / Collagenases)

MMP such as MMP – 8 have been isolated from the dentin – pulp complexas well as from the odontoblasts, and are known to play a crucial role in Dentinogenesis as well as caries progression modulation. Studies reveal that level of MMP – 8 is higher in pulpitis than compared to a healthy pulp (10). A study by Aguirre-López EC et al, showed that an increase in the duration of cold test response was associated with an elevated levels of MMP-8 in teeth diagnosed with pulpitis, thereby highlighting that a higher level of MMP – 8 is found in irreversible pulpitis than in reversible pulpitis (11).

Matrix metalloproteinase – 9 (MMP – 9)

Among all the MMPs studied in inflamed pulpal tissue (MMP 1, 2, 3, 8 and 9), MMP – 9 is regarded as a key metalloproteinase involved in the breakdown of the pulpal tissue. Various studies have found that the concentration of MMP – 9 is significantly different in pulp tissue during different stages of pulpal inflammation, with higher concentration seen in symptomatic irreversible pulpitis than in teeth with normal pulps (12). These studies thereby showed that evaluating the level of MMP – 9 could be used as a potential discriminatory marker for pulpal diagnosis and also as a predictor for the outcome of pulpotomy (12).

Substance – P (SP)

Substance P (SP), a neuropeptide secreted by the sensory nerve endings and as a response to neurogenic inflammation of the pulp by various inflammatory cells such as neutrophils, monocytes/macrophages, eosinophils, lymphocytes, and dendritic cells. SP upon release is attributed to control the blood flow and regulation of inflammation and tissue repair.
Substance P can serve as a biomarker to accurately diagnose and link the inflammatory state of the pulp with the pain experienced by the patient. A systematic review by Arun N et al, showed that there is a significant increase in SP level in teeth with irreversible pulpitis than compared to healthy teeth (13).

Calcitonin gene-related peptide (CGRP)

CGRP is a neuropeptide directly involved in Neurogenic pulp inflammation. This neuropeptide is released from the terminal end of unmyelinated C fibers and possibly other pulpal cells, and may contribute to inflammatory and vascular changes that lead to inflammatory or necrotic changes in pulpal tissue. Increased production of CGRP takes place in teeth with irreversible pulpitis than compared to the healthy pulp. Various studies have shown basal level CGRP in normal healthy pulpal tissues whereas an increase in their level in irreversible pulpitis.

Osteocalcin

Osteocalcin (OCN) is a reparative molecule commonly expressed in response to injury of the dental pulp. Osteocalcin is considered a late-stage marker of odontoblast differentiation. Macrophages which are predominant in an inflamed pulp may express Osteocalcin during their trans differentiation to

osteoblasts and thereby, signifying that large quantity of these molecules are secreted from the odontoblasts during reactionary Dentinogenesis in response to the pulp inflammation. A study by Abd-Elmeguid A et al showed that OCN expression increased in inflamed pulps compared with normal pulps (14). The study indicated that concentration of OCN is higher in reversible pulpitis than in irreversible pulpitis, suggesting its potential role in dental pulp repair and regeneration. The level of OCN is found to be reduced as the inflammation progresses from reversible to irreversible, with the expression of catabolic molecules like IL-1a and IL-1b, thus further proving its role in dental pulp repair (14). But a recent study by Kritikou K et al showed an increased level of various biomarkers including OCN to be increased in irreversible pulpitis as compared to healthy dental pulp (15). Another study by F Benetti et al showed an increase OCN level over time after vital tooth hydrogen peroxide bleaching, implicated its role in tissue repair process after bleaching induced pulpal irritation (16).

Human b-defensins (hBDs)

hBDs is a cationic antimicrobial peptide produced naturally in the human body and play an important role such as broad-spectrum antimicrobial peptide, inflammatory inhibitor, regulator for cellular differentiation, anti-cancer activity etc. In addition, it is shown to play vital role in dental pulp tissue also, with increased levels of hBD seen in inflamed dental pulp. So far, 4 human b-defensins (hBD-1, -2,-3, and-4) have been isolated. A study by Paris S et al showed that basal hBD 1 and hBD 4 expression increases as the inflammation of pulp increases (17)

Interleukins (IL)

Cytokines such as IL, Interferons, Tumor necrosis factors (TNF) and Chemokines are small glycoproteins that are produced and secreted by a wide range of immune and non-immune cells and affect many interactive processes between these cells such as Proliferation, Differentiation, cell requirement, apoptosis and etc. Cytokines are generally excellent markers of inflammation. Various studies have shown an elevated levels of IL in inflamed pulp tissue (18). Higher levels of ILs in the context of pulpal inflammation can stimulate the pulp cells to secret MMPs, thereby triggering extracellular matrix degradation in the pulp chamber (18). ILs can have both inflammatory (IL-2, IL-6, IL-8) and anti-inflammatory (IL-4, IL-10, IL-13) effects, thereby acting as sensitive inflammation modulators. Levels of IL-1α shown to be significantly higher in both reversible and irreversible pulpitis, with highest concentration in irreversible pulpitis (18). It is also seen that significantly higher levels of IL-2 in asymptomatic caries exposure cases/ mildly inflamed pulpal tissue than compared with both irreversible pulpitis and normal healthy pulp (19). This indicates that the pulp has initiated immunological repair process against the caries lesion.

RANKL and Osteoprogerin (OPG)

RANKL and OPG are signaling molecules regulating bone metabolism and immune function. RANKL, found in various dental tissues – odontoblasts, dental pulp cells, periodontal ligament etc, promotes bone resorption by binding to clastic cells, while OPG inhibits this process. Their expression in apical periodontitis indicates a role in periapical lesion development, with pro-inflammatory cytokines inducing RANKL and OPG countering lesion expansion. A recent study showed statistically significant higher levels of RANKL and OPG in both asymptomatic and symptomatic apical lesions compared to healthy periapical tissues (20). RANKL/OPG ratio has been proposed as an indicator of apical lesion progression. The OPG/RANKL/RANK system plays a key role in root resorption during orthodontic tooth movement and following traumatic injury. Therefore, assessing the levels of OPG and RANKL in GCF can provide insight into the degree of root resorption during orthodontic tooth movement. However, these are predominantly mediators of bone remodeling, thus not specific for root resorption.

Tartrate-resistant acid phosphatase (TRAP)

Also called acid phosphatase, is a glycosylated monomeric metalloprotein enzyme. In addition to bone matrix degradation products released by active osteoclasts, it acts as a biomarker for osteoclastic activity and bone resorption in periradicular or periapical lesions, including abscesses and cysts. Higher levels of TRAP are associated with the progression of bone destructive diseases. Studies show a higher level of TRAP in symptomatic apical lesions, suggesting their role in progressive lesions (20). TRAP-5 has a diagnostic potential for Symptomatic apical periodontitis, representing a potentially and most reliable candidate biomarker for evaluating the apical periodontitis progression.

Deoxypyridinoline (DPD)

Pyridinoline cross-links are collagen degradation products that belong to the family of C- and N-telopeptides. This group also includes pyridinoline and deoxypyridinoline (DPD), with DPD being predominantly found in bone. These molecules are released into the bloodstream as a result of bone resorption and collagen matrix breakdown, making them specific markers for bone resorption. Since DPD is only produced during the resorption of mature bone matrix, elevated levels of DPD are often associated with bone resorption, particularly in conditions such as severe periapical lesions.

Dentin Sialophosphoprotein (DSPP)

Dentin Sialoprotein (DSP) and Dentin Phosphoprotein (DPP) are components of Dentin Sialophosphoprotein (DSPP), crucial dentin-specific non-collagenous proteins. Produced by dental pulp cells into the dentinal matrix, these proteins are most abundant during root resorption, unlike other markers typically linked with physiological bone resorption. Mah and Prasad were pioneers in detecting these biomarkers in the gingival crevicular fluid (GCF) of patients experiencing root resorption, particularly in cases of orthodontically induced resorption. Elevated DSP levels in teeth during both physiological and orthodontically induced root resorption suggest that measuring DSP in GCF could be a valuable biomarker for monitoring root resorption. Studies also show higher concentration of DSP and DPP in the GCF of patients with root resorption than in healthy individuals, with highest concentration in severe resorptions (>2mm root shortening) than compared to mild resorption (21). This demonstrates that these biomarkers are suitable for monitoring root resorption prior to kts appearance on radiographs.

microRNS (miRNAs)

These are predominantly biomarkers in the diagnosis of certain cancers, autoimmune diseases and inflammatory diseases, but several microRNAs have been found to be found in the GCF during orthodontically induced root resorption (21). As the extent of orthodontic root resorption increases, there is a decrease in the expression of these molecules in the GCF of Orthodontic patients experiencing root resorption. Transfection of miR-155 mimic has been shown to significantly suppress osteoclast formation, while transfection of miR-155 inhibitor has been observed to significantly increase osteoclast formation, thus revealing the inhibitory effect of miRNA on osteoclast differentiation (22).miR-21 promoted orthodontic tooth movement by regulating the RANKL/OPG balance as per certain studies (23). miRNAs have also been noticeable in other dental conditions such as pulpitis.

C Reactive Protein (CRP)

Biomarkers such as CRP are found to be linked to acute inflammation and are said to be indicative of recent or acute phase of periapical lesions. CRP levels can increase significantly as the severity of apical periodontitis increases.

Forkhead box P3 (FoxP3) transcription factor

The progression and outcome of apical periodontitis are believed to depend on the balance between the host's pro-inflammatory and anti-inflammatory responses, a process that is regulated by different subsets of CD4+ T helper cells, such as Th1, Th2, Th17, and Treg cells. Accumulating evidence has revealed the advantageous role of Treg cells in reducing the over activity of periapical inflammatory response. The number of Treg cells was found to be relatively low during acute phase of the periapical lesion (day 7 to 21) and then increased significantly by day 35 (i.e; chronic phase) (24). FoxP3 is the best and most reliable immunohistochemical marker for Treg cells (T regulatory cells).

Mesenchymal Stem Cell Marker

Mesenchymal stem cells (MSCs) have been investigated for their role in the development of various diseases, including periapical cysts and periapical granulomas. Periapical cysts are an inflammatory response to endodontic infection and are considered a progression of chronic apical periodontitis, although not all cases of chronic apical periodontitis led to cyst formation. Studies have identified osteogenic cells in certain periapical lesions that have the potential to differentiate into osteoblastic cells, promoting healing of the surrounding periapical bone. Various studies showed presence of MSC in periapical lesions such as Periapical granuloma and cysts (25). It is important to note that stem cells can remain in a quiescent state within their niche and may be activated or released through unknown mechanisms, such as the acute inflammatory infiltrate during periapical inflammation, which can lead to notable changes in their behavior, thus acting as potential biomarker candidate.

Chair side tests and assays

The development of evaluation tools for clinical use is an area that requires further attention. Ideally, any chairside tests used in endodontics to detect local biomarkers should be simple, affordable, and easy to use. For instance, rapid membrane-based lateral flow immunoassays, commonly used in periodontology, could also be adapted to identify endodontic biomarkers. These tests offer a binary result, indicating whether a specific biomarker or molecule is present above a certain detection threshold. Such assays could be valuable for identifying biomarkers with predictive potential for determining the success of vital pulp treatment. Another simple and potential chair side assess have been proposed in the form of rapid fluorescence tests in conjunction with paper points. Biosensors are described as devices used for biomarker detection in point-of-care environments. Advanced diagnostic tests for more complex conditions could utilize microfluidics and electrical engineering techniques to analyze metabolites and other molecules in small volumes of patient-derived biological samples. Technologies like Lab-on-a-Chip and Lab-on-a-Disk systems are examples of such innovations. Lab-on-a-Chip (LOC) devices are microdevices that integrate microfluidic technology with electrical and/or mechanical functions to analyze very small fluid volumes (26). These compact and portable devices function like miniaturized laboratories, capable of performing various laboratory tasks such as sample manipulation and biomarker detection, all while using minimal specimen quantities. LOCs have been employed for the detection and quantification of a range of cells, biomarkers, pathogens, and contaminants in small fluid samples. Lateral Flow Assays (LFAs), also known as "test strips," are among the most commonly used platforms for microfluidic diagnostics. They allow for the detection of biomarkers in liquid samples without the need for sophisticated equipment or trained personnel. The routine use of these technologies could eliminate the need for centralized, time-consuming laboratory testing, allowing for immediate clinical decision-making at the point of care. By leveraging these advancements, valuable microbiological, immunological, or metabolic data can be obtained, aiding in accurate diagnosis and enabling more effective clinical decision-making to develop an appropriate treatment plan.

Conclusion

Endodontists continue to have differing opinions on matters like vital pulp therapy versus conventional root canal treatment, or retreatment versus periapical surgery. In these scenario, inflammatory biomarkers play a diverse and broad role as a potential key factor in determining treatment plans by assisting in diagnosis and prognosis. Mainly, detecting mediators may allow us to infer the state of inflammation. Butclinical challenges such as determining the proper tissue fluid sample to analyze, establishing a precise inflammatory threshold, and efficiently delivering results at the chairside must be overcome.

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