EVALUATION OF ORAL NEUTROPHIL LEVELS
AS A QUANTITATIVE MEASURE OF PERIODONTAL
INFLAMMATORY LOAD IN PATIENTS WITH SPECIAL NEEDS

By

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ABSTRACT

Evaluation of Oral Neutrophil Levels as a Quantitative Measure of Periodontal Inflammatory Load in Patients with Special Needs

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Masters of Science in Paediatric Dentistry, 2012

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Purpose: To validate and assess the feasibility of using an assay of oral neutrophils to measure periodontal inflammation in uncooperative patients with special needs.

Methods: Periodontal examination and neutrophil counts derived from oral swabs were performed on patients with special needs having comprehensive dental treatment under general anaesthesia (GA). The conventional periodontal measurements were compared to neutrophil levels while patients were under GA, and later at their recall examination.

Results: Forty-nine patients were assessed under GA and 30 (61%) returned for recall examination. Spearman’s correlation allowed for comparisons between periodontal parameters and oral neutrophil counts. Despite limited cooperation, it was possible to acquire neutrophils (using swabs) for all patients that presented for recall examination in the ambulatory dental clinic.

Conclusions: Oral neutrophil levels correlated significantly with conventional parameters of gingival inflammation and may serve as a standardized method for clinical assessment of periodontal diseases in the special needs population.
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<td><strong>ABTS:</strong> 2, 2’ – azinobis (3-ethylbenzothiazoline-6-sulphonic acid)</td>
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<tr>
<td><strong>ANCOVA:</strong> Analysis of covariance</td>
</tr>
<tr>
<td><strong>BOB:</strong> Bleeding on brushing</td>
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<tr>
<td><strong>BOP:</strong> Bleeding on probing</td>
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<td><strong>CAL:</strong> Clinical attachment loss</td>
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<td><strong>CI:</strong> Calculus index</td>
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<td><strong>CRP:</strong> C reactive protein</td>
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<td><strong>DM:</strong> Decision maker</td>
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<td><strong>GA:</strong> General anaesthesia</td>
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<td><strong>GCF:</strong> Gingival crevicular fluid</td>
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<td><strong>GS:</strong> Gold standard</td>
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<tr>
<td><strong>ICC:</strong> Intra-class correlation coefficient</td>
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<tr>
<td><strong>M:</strong> Mobility</td>
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<td><strong>MGI:</strong> Modified gingival index</td>
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<td><strong>MMP:</strong> Matrix metalloproteinase</td>
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<td><strong>MPO:</strong> Myeloperoxidase</td>
</tr>
<tr>
<td><strong>NADPH:</strong> Nicotinamide adenine dinucleotide phosphate oxidase</td>
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<tr>
<td><strong>OMR:</strong> Orogranulocytic migratory rate</td>
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<td><strong>PD:</strong> Probing depths</td>
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<td><strong>PI:</strong> Plaque index</td>
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<tr>
<td><strong>PMN:</strong> Polymorphonuclear leukocyte or neutrophil</td>
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<tr>
<td><strong>PMNs:</strong> Polymorphonuclear leukocytes or neutrophils</td>
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<tr>
<td>$r_s$: Spearman correlation coefficient</td>
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<tr>
<td><strong>TNFα:</strong> Tumor necrosis factor alpha</td>
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<td><strong>VAS:</strong> Visual analog scale</td>
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INTRODUCTION AND STATEMENT OF INTEREST

Patients with special needs have a high prevalence of oral disease (Scott, March, & Stokes, 1998; Lopez del Valle, Waldman, & Perlman, 2007; Dellavia, Allievi, Pallavera, Rosati, & Sforza, 2009; Anders & Davis, 2010), which can be difficult to diagnose with the conventional instruments that are used to detect disease in the dental office, where a periodontal examination may be impossible due to lack of patient cooperation. Literature to propose or support the use of alternative strategies for diagnosis of gingival or periodontal health status in the special needs population is lacking (Hennequin, Faulks, & Roux, 2000). The lack of appropriate diagnoses of gingival and periodontal diseases prevents the delivery of optimal treatment. Therefore, periodontal problems could go undetected and untreated. This is especially relevant when recognizing oral health as an important component of general health and well-being (Boehm & Scannapieco, 2007; Brennan, Spencer, & Roberts-Thomson, 2007), and the increased potential risk of pulmonary infections in special needs patients with poor oral hygiene (Scannapieco, 1999; Scannapieco, 2005b; Scannapieco, 2006; Sigal & Sigal, 2006).

Periodontal diseases are a group of inflammatory disorders characterized by progressive destruction of the periodontium, specifically the tissues surrounding and supporting the teeth (Armitage, 1999). The destruction of tissue observed in the presence of periodontitis is a consequence of an interaction between the host inflammatory response and pathogenic bacteria within the periodontal crevicular space (Deas, Mackey, & McDonnell, 2003; Van Dyke, 2008; Sanz & van Winkelhoff, 2011). This excessive inflammation leads to a
disruption of the normally homeostatic relationship between bone and periodontal ligament formation and breakdown - in favour of breakdown. Once this homeostatic relationship is altered as noted above, the eventual result is that of loss of tissues supporting the teeth, and ultimately, loss of teeth. Disease susceptibility, progression, severity, and response to treatment are mostly determined by host-based factors (Kinane, Peterson, & Stathopoulou, 2006). Periodontal disease significantly increases the risk of tooth loss resulting in loss of masticatory function, malnutrition that affects general health, and an overall decrease in quality of life (Boehm & Scannapieco, 2007; Brennan, Spencer, & Roberts-Thomson, 2007).

Clinical parameters that are currently used for the assessment of gingival health status include: erythema, swelling or enlargement of gingival contours due to edema or soft tissue hyperplasia, plaque and calculus accumulation, periodontal probing depths, bleeding on probing, clinical attachment loss, gingival recession, furcation involvement, tooth mobility, and radiographic alveolar bone loss (Haffajee, Socransky, & Goodson, 1983; Mariotti, 1999; Pihlstrom, 2001). Though considered to be the gold standard in periodontal disease diagnosis, these parameters are noted to have numerous limitations, the most important being the fact that these measures do not necessarily indicate whether a patient with periodontitis has active or inactive disease (i.e. will the disease continue to progress without treatment; Haffajee et al., 1991; Armitage, 1996). Acknowledging the limitations of the currently used clinical measures of periodontal health status emphasizes the necessity for pursuit of other and better diagnostics for periodontal disease (Apsey, Kaciroti, & Loesche, 2006).
Polymorphonuclear leukocytes (PMNs) or neutrophils, are a major cellular element of the innate immune system (Lavelle, 1992; Hart, Shapira, & Van Dyke, 1994), and are vital for the host’s immune response to virulent bacteria (Van Dyke & Hoop, 1990). Neutrophils play a major role in the host response to invading periodontal pathogens (Hart, Shapira, & Van Dyke, 1994), and the gingival sulcus is the major site of entry of leukocytes into the oral cavity (Sharry & Krasse, 1960). The PMN is able to detect infection, migrate to the site of disease, destroy pathogens, and influence bacterial growth and colonization in periodontal tissues (Deas, Mackey, & McDonnell, 2003). Neutrophils have been shown to increase in quantity in proportion to gingival inflammation (Attstrom, 1970; Schroeder, 1973), and the protection conferred by the host response effectively defends against the bacterial insults that constantly threaten the health status of the periodontium in the majority of the population (Deas, Mackey, & McDonnell, 2003; Schenkein, 2006). However, the protection provided by the PMN is not without consequence. The PMN’s bactericidal or killing mechanisms also have the potential to damage extracellular tissues via the release of granular constituents containing degradative enzymes called matrix metalloproteinases (MMP), for example, MMP-8, also known as collagenase, myeloperoxidases (MPO), and reactive oxygen species (Van Dyke & Hoop, 1990; Lamster, 1992; Deas, Mackey, & McDonnell, 2003).

The use of PMN quantification to assess periodontal disease status and responses to treatment was first proposed in 1978 (Raeste & Aura, 1978), an idea which seems useful when considering the effect of PMNs on periodontal tissues, and the fact that their levels are naturally elevated during disease (Attstrom, 1970; Schroeder, 1973), and are likely proportional to the extent of disease activity (Attstrom, 1970; Schiött & Löe, 1970).
A simple method for measurement of oral PMNs has been developed and involves the use of a non-invasive oral rinse assay. This was validated at the University of Toronto’s Department of Periodontology in 2006, and demonstrates promise in allowing the clinician to monitor the activity and severity of periodontal disease as well as its progression (Bender, Thang, & Glogauer, 2006). However, the use of the oral rinse assay is impractical in patients who lack the cooperation and/or coordination required to reliably rinse and expectorate.

This research study will evaluate a novel technique for oral neutrophil quantification, using a PMN assay, as a quantitative measure of periodontal inflammatory load in uncooperative patients with special needs.

GOALS

1. To correlate oral neutrophil counts obtained by the PMN assay with conventional clinical parameters of periodontal health status in uncooperative patients with special needs.

2. To assess the feasibility of using the PMN assay to provide objective and quantitative data so that gingival inflammation can be quantified and monitored in uncooperative patients with special needs.
REVIEW OF THE LITERATURE

Definitions and Prevalence of Disability

Patients with special health care needs are defined as those with a, “physical, developmental, mental, sensory, behavioural, cognitive, or emotional impairment or limiting condition that requires medical management, health care intervention, and/or use of specialized services or programs” (American Academy of Pediatric Dentistry Council on Clinical Affairs [AAPD], 2011/12). Disabilities can be congenital or acquired, as well as either visible (i.e. physical), or invisible (e.g. mental). The International Classification of Functioning, Disability, and Health (ICF), defines disability as a broad term that looks at the interaction of health conditions, environmental, and personal factors on body functions, individual activities, and participation in society (World Health Organization [WHO], 2011). Developmental disability is defined as a mental or physical impairment with onset during the developmental period from birth to 22 years of age, and results in significant functional limitations in three or more areas of major life activities, such as self-care, language, mobility, or capacity for independent living (Accardo & Whitman, 2012). Approximately 1.85 million people or about 15% of the population in Ontario have a disability (Ontario Government, 2010), and disability is reported by about 4.4 million Canadians (14.3%; Government of Canada, 2010). The World Health Organization estimates that over one billion people, or 15% of the world’s population, have some form of disability (WHO, 2011).
Oral Health Care Needs for Patients with Special Needs

Oral disease, including caries and periodontal disease, is significantly prevalent in the special needs population (Scott, March, & Stokes, 1998; Lopez del Valle, Waldman, & Perlman, 2007; Dellavia, Allievi, Pallavera, Rosati, & Sforza, 2009; Anders & Davis, 2010). Oral health is noted to be especially poor in patients who are unable to cooperate for routine dental care (Anders & Davis, 2010), due to movement disorders, cognitive problems or any number of disabilities. Data are limited regarding oral health care in patients with severe developmental delays. These patients need more supervision and are reliant on caregivers for their daily oral hygiene routine. Further, they are dependent on dentists who are comfortable in treating them despite their special needs, medical health complexity, and lack of cooperation (de Jongh, van Houtem, van der Schoof, Resida, & Broers, 2008).

Unfortunately, persons with disabilities experience significant disparities and unmet oral health care needs from dental professionals (Scott, March, & Stokes, 1998; Loeppky & Sigal, 2007; Sigal, 2009). Reasons cited for this disparity include lack of training in the dental curriculum leading to lack of familiarity with this population, belief that special equipment is required, lack of cooperation or communication leading to difficulty in diagnosis, increased time involvement for treatment, or lack of reimbursement for dental services provided through government programs (Fenton, Hood, Holder, May, & Mouradian, 2003; Loeppky & Sigal, 2007; Lopez del Valle, Waldman, & Perlman, 2007; Koneru & Sigal, 2009). The demands for oral health care are only going to increase in this population with the average lifespan increasing due to advances in medical care, and continued deinstitutionalization and integration of persons with special needs into the community (Scott, March, & Stokes, 1998;
Sigal, 2009). Thus, appropriate diagnosis and provision of dental treatment to adult patients that are uncooperative due to varying degrees of developmental delay is a persistent challenge in dentistry (Ananthanarayan, Sigal, & Godlewski, 1998).

The Mount Sinai Hospital Dental Program for Persons with Disabilities

The Mount Sinai Hospital Dental Program for Persons with Disabilities, the largest of its kind in Canada, was established in 1975 and provides comprehensive oral health care to patients with special needs in a hospital setting (Sigal, 2010). Patients are referred by their family physician, dentist, case support worker, or allied institution. The hospital based program plays a vital role in providing dental care to patients who are unable to receive treatment in their local community. The overall need for this program is reflected by the current waiting list for dental treatment under GA, which is approximately twelve months following the initial consultation (Park & Sigal, 2008). The Program also serves to educate undergraduate and graduate students attending the University of Toronto’s Faculty of Dentistry in conjunction with the Department of Paediatric Dentistry, to familiarize students with this population so that they are comfortable in treating patients with special needs in their future community practice (Sigal, 2010). Dental recall examinations for patients with special needs are provided by dental students supervised by staff, as well as hospital dental residents. Dental treatment is provided by hospital dental staff and residents in the ambulatory dental clinic as well as under GA in the operating room. However, despite attempts to manage behaviour in the clinical setting, many patients with special needs still require dental treatment under GA due to poor cooperation (Hennequin, Faulks, & Roux, 2000; Petrovic, Markovic, & Peric, 2011). The Mount Sinai Hospital Dental Clinic has
provisions for uncooperative patients to have comprehensive dental care safely and predictably in one treatment session (Ananthanarayan, Sigal, & Godlewski, 1998), followed by de-sensitization to the dental clinic via subsequent dental recalls without sedation. However, there are still important limitations related to examination, diagnosis, and treatment in this population that will be described later.

Classification of Periodontal Diseases

Periodontal diseases are a group of inflammatory disorders that may be inherited or acquired, and are characterized by progressive destruction of the tissues that surround and support the teeth, known as the periodontium (Armitage, 1999). The periodontium is comprised of root cementum, the periodontal ligament, alveolar bone, and its overlying mucosa (Nanci & Bosshardt, 2006). The prevalence of periodontal diseases as reported in the literature varies between 14 to 65% depending on the definitions used and the population studied (Costa et al., 2009). The most common periodontal diseases are grouped under two broad categories of conditions, called gingivitis and periodontitis (Armitage, 1999).

Gingivitis is defined as inflammation of the gingiva associated with teeth that do not demonstrate attachment loss. Gingivitis is further divided into those conditions which are plaque-induced and those that are not (Armitage, 1999). ‘Plaque-induced’ gingivitis is a consequence of the interaction between the pathogens within the dental plaque or biofilm at the tooth/gingival interface, and the host tissues and inflammatory cells within them (Armitage, 2004). This interaction between the plaque and host is influenced by local and epigenetic factors such as smoking and plaque, medical problems including but not limited to
diseases such as diabetes mellitus, medications including anticonvulsants, immunosuppressants, oral contraceptives, and calcium channel blockers, and malnutrition (Armitage, 1999; Mariotti, 1999; Kinane & Marshall, 2001). ‘Non-plaque-induced’ gingival conditions are caused by specific bacterial, viral, fungal, or genetic factors, or can imply the presence of an underlying medical condition (Armitage, 1999). Clinical signs of gingivitis include enlarged gingival contours due to edema or fibrosis, change in color (erythema), elevated sulcular temperature, bleeding on stimulation, and increased gingival exudate. These signs are associated with stable attachment levels on a periodontium that may or may not be reduced (Mariotti, 1999). In gingivitis, inflammation is limited to the gingiva and may be reversed by removing the etiological factors (i.e. plaque; Löe, Theilade, & Jensen, 1965). Persistent inflammation may have a role in the progression to periodontal attachment loss (Mariotti, 1999).

Periodontitis is a condition characterized by gingival inflammation of the periodontium, as with gingivitis, but is also associated with the irreversible destruction of connective tissue apical to the cemento-enamel junction, referred to as periodontal attachment loss (Ranney, 1991). Periodontitis is divided into other categories including chronic, aggressive, or as a manifestation of systemic (i.e. non-oral) disease. These categories are distinguished further based on the extent and severity of disease as follows. Chronic periodontitis can be localized (less than 30% of sites are involved), or generalized (more than 30% of sites are affected). Severity is characterized by the amount of clinical attachment loss (CAL) as measured by a periodontal probe, as slight (1-2 mm CAL), moderate (3-4 mm CAL), or severe (≥ 5 mm CAL; Armitage, 1999). The primary etiological agent appears to be gram negative bacteria,
though the exact spectrum of pathogens contained within the biofilm which initiates periodontitis is not firmly established. Moreover, it has been suggested that these suspected pathogenic bacteria are necessary but not sufficient to trigger periodontitis (Socransky & Haffajee, 1992; Van Dyke, 2008). The tissue destruction in periodontitis is a consequence of an imbalance in the homeostatic relationship between tissue resorption and tissue genesis, such that tissue resorption is favoured under the influence of an unregulated inflammatory response to the pathogenic bacteria within the periodontal crevicular space and as alluded to above, other non-microbial factors (Deas, Mackey, & McDonnell, 2003; Van Dyke, 2008; Sanz & van Winkelhoff, 2011). Susceptibility to disease, progression, severity, and response to treatment are determined mostly by host-based factors (Kinane, Peterson, & Stathopoulos, 2006). The clinical presentation of chronic periodontitis includes loss of alveolar bone, bleeding upon probing, tooth mobility, and ultimately, if the disease is left untreated, tooth loss (Pihlstrom, Michalowicz, & Johnson, 2005). In addition to effects on the periodontium caused by periodontitis, it is also noteworthy that as a consequence of the disease and its treatment, those with periodontal disease have an increased risk for root caries (Boehm & Scannapieco, 2007). Therefore, periodontitis increases the risk for loss of teeth due to the disease itself, but in the longer term tooth loss is also due to an increased risk for root caries, a form of tooth decay that proceeds rapidly and can have rather devastating effects on the teeth. Premature loss of teeth can lead to several problems including but not limited to the following: loss of masticatory function, malnutrition that could worsen the general health, speech difficulties, and an overall decrease in quality of life (Boehm & Scannapieco, 2007; Brennan, Spencer, & Roberts-Thomson, 2007).
The Oral and Systemic Health Connection

The importance of oral health with respect to overall health and well-being has been recognized by an increasing body of literature illustrating the association between periodontal health and general health in conditions such as diabetes mellitus, cardiovascular disease, and pulmonary infections (Scannapieco, 1999; Rutkauskas, 2000; Kinane & Marshall, 2001; Teng et al., 2002; Scannapieco, 2005a; Azarpazhooh & Leake, 2006; Raghavendran, Mylotte, & Scannapieco, 2007; Kuo, Polson, & Kang, 2008; Azarpazhooh & Tenenbaum, 2012a). The connection lies at the thin and permeable gingival sulcus, which serves as a barrier between the oral environment and underlying tissues. In fact, this is the only area in the human body where the integumentary system is actually penetrated by another structure. The junctional epithelium is highly porous, as epithelial cells are connected by desmosomes and few gap junctions, resulting in large fluid-filled intracellular spaces (Bosshardt & Lang, 2005). In the presence of inflammation, this barrier may be penetrated easily by microorganisms that enter the underlying vasculature, thereby presenting a biological challenge to other parts of the body (Scannapieco, 2005a). Efforts to establish a causal link between oral and systemic diseases appear to be fading in favor of research focusing on the likely similar inflammatory mechanisms which underlie both oral and non-oral disease pathways (Kantarci & Van Dyke, 2005; Teles & Wang, 2011; Vaishnava, Narayan, & Fuster, 2011), where the term ‘Inflammatory Syndrome’ may better and more appropriately describe the connection and commonalities between oral and general health and disease mechanisms (Azarpazhooh & Tenenbaum, 2012b).
Periodontal Disease: An Inflammatory Syndrome

Periodontal diseases are manifestations of an inflammatory process. Inflammation is defined as, “a complex reaction to injurious agents such as microbes and damaged, usually necrotic, cells that consist of vascular responses, migration and activation of leukocytes, and systemic reactions” (Kumar, Abbas, & Fausto, 2005). Thus, the process of inflammation leads to dysregulation of the health-associated homeostatic relationships between tissue destruction and repair. Inflammation begins as an acute reaction of rapid onset and of relatively short duration, and is characterized by fluid exudate and leukocyte emigration into the periodontal pocket surrounding the affected tooth or teeth, consisting mostly of PMNs. In contrast, chronic inflammation occurs over a longer period of time and is mediated by cells such as lymphocytes and macrophages, as well as pathophysiological responses such as blood vessel proliferation and fibrosis of the surrounding tissues. Inflammation ceases when the invading agents and host mediators are removed (Kantarci & Van Dyke, 2005).

Pathogenesis of Periodontal Diseases

The tissue destruction that is noted in periodontal disease is currently thought to occur as a result of an interaction between the pathogenic bacteria residing in the periodontal pocket and the host inflammatory response (Deas, Mackey, & McDonnell, 2003). Constituents of the microbial biofilm stimulate increased inflammatory infiltrate to the gingival crevice. This infiltrate includes many serum components including markers of inflammation as well as particular cell types such as neutrophils, macrophages, and lymphocytes (Kim & Amar, 2006). Non-microbial components of the gingival crevicular fluid that are being affected by inflammation may contain microbe-derived factors including lipopolysaccharides. These
agents can also activate the host immune response so that various pro- and anti-inflammatory cytokines are produced. The pro-inflammatory cytokines include tumor necrosis factor alpha (TNFα), prostaglandins (especially prostaglandin E₂), as well as various hydrolytic enzymes, some of which are MMPs. In addition, T lymphocytes can be stimulated to release cytokines and lymphotoxin. These substances possess intense pro-inflammatory and catabolic activities, causing periodontal tissue breakdown, typically mediated by the MMPs noted above. It is thought that the presence of these inflammatory molecules and cells marks the transition from gingivitis to periodontitis, and PMNs are thought to be especially important in the latter (Page, 1998; Nussbaum & Shapira, 2011). Basically, the severity and extent of this destruction depends on dynamic interactions between the host inflammatory response and the destructive pathobiological responses around the affected teeth (Lee, Aitken, Sodek, & McCulloch, 1995; Kim & Amar, 2006; Jin, 2011).

Inflammatory Load: A Novel Term in Dentistry

We propose that the inflammatory infiltrate as described above, which consists mostly of PMNs (Van Dyke & Hoop, 1990), and leads to the inflammatory cascade, may be described as the “inflammatory load” of an individual. The “inflammatory load” has been described in the medical literature (O’Donovan et al., 2011; Bassiouni, Naidoo, & Wormald, 2012), as a measure of inflammatory markers in blood or other tissues (Jin et al., 2010). In the realm of dentistry and specifically in this study, this term would reflect the inflammatory burden of the individual in the presence of oral disease, which is modifiable by removing the stimulating factors with treatment (e.g. pathogenic bacteria within the oral biofilm), as will be discussed later.
The Role of Neutrophils in the Host’s Immune Response

Neutrophils are a major cellular element of innate immunity (Lavelle, 1992; Hart, Shapira, & Van Dyke, 1994), are the predominant leukocytes in the blood (Miyasaki, 1991), and are vital in the host’s immune response to virulent bacteria (Van Dyke & Hoop, 1990). With regard to differentiation and development of PMNs, their precursors are found in the bone marrow as stem cells. The process by which leukocytes are produced is called leukopoiesis. Cytokines such as interleukins and colony-stimulating factors regulate this process, and allow stem cells to develop into a myeloid stem cell or lymphoid stem cell. The myeloid pathway gives rise to the neutrophils, as well as monocytes, basophils, and eosinophils. Notably, the myeloid stem cell passes through five precursor stages in the process of differentiation and maturation into a PMN: myeloblast, promyelocyte, neutrophil myelocyte, neutrophilic metamyelocyte, and then neutrophilic band cell. The lymphoid pathway results in T- and B-lymphocytes. After the stem cells differentiate and mature into PMNs, they are released into the bloodstream. Neutrophil development takes about 2 weeks, and approximately one hundred billion are produced daily (Deas, Mackey, & McDonnell, 2003). Neutrophils then migrate and adhere to the endothelium of blood vessels before passing into the connective tissues as extravascular cells (Van Dyke & Hoop, 1990). Under normal conditions of health, 90% of the body’s PMNs reside in the bone marrow. Many of the daily produced neutrophils will undergo apoptosis prior to leaving the bone marrow. For neutrophils that are released, the half-life ranges from 6 to 9 hours (in circulation), to 1 to 4 days (in tissue; Trowbridge & Emling, 1997; Deas, Mackey, & McDonnell, 2003). Typically, after initiation of bacterial colonization (and invasion in some cases), PMNs will migrate to the site of infection. Neutrophils pass from the connective tissue to the gingival sulcus via the intercellular spaces
of the junctional epithelium (Attstrom, 1970; Schroeder, 1973). It is here that cells enter the oral cavity, continue to function without impairment, and can be collected for analyses (Klinkhamer, 1968).

Neutrophils are attracted to the site of infection in response to chemotactic molecules activated by invading microbes, such as complement proteins. The bacteria are then identified and engulfed by the PMN into a phagosome. The cell membrane of the phagosome fuses with the membranes of cytoplasmic granules, resulting in the release of granule contents (called “degranulation”), into the phagosome as well as the extracellular environment in order to kill the invading microbes. It is important to note at this point that the PMN cytoplasm contains several types of granules or specialized lysosomes that are classified as primary, secondary, and tertiary granules. The primary granules contain acid hydrolases, neutral proteases, cationic proteins, myeloperoxidase, and lysozymes. The secondary granules contain lysozymes, lactoferrin, and collagenase. Finally, the tertiary granules contain cytochrome b and alkaline phosphatase (Van Dyke & Hoop, 1990). These factors play an important role in bacterial killing but can also be used as markers of PMN activity, quantity, and inflammation (Cao & Smith, 1989).

The Role of Neutrophils in Periodontal Disease

Neutrophils play a major role in the host response to invading periodontal pathogens (Hart, Shapira, & Van Dyke, 1994). The gingival sulcus is the major site of entry of leukocytes into the oral cavity (Sharry & Krasse, 1960). In fact, about 90% of leukocytes isolated from gingival crevicular fluid are PMNs (Woolweaver, Koch, Crawford, & Lundblad, 1972). The
PMN is able to detect infection, migrate to the site of disease, destroy pathogens, and influence bacterial growth and colonization in periodontal tissues (Deas, Mackey, & McDonnell, 2003). Neutrophils have been found in the gingival crevice of clinically healthy gingival tissues (Attstrom, 1970; Raeste, Tapanila, & Tupakka, 1977), and have been shown to increase in quantity with gingival inflammation (Attstrom, 1970; Schroeder, 1973), and when comparing dentate to edentulous patients (Calonius, 1958). The number of granulocytes in saliva have also been shown to increase prior to the appearance of clinical gingivitis (Friedman & Klinkhamer, 1971), and line the junctional epithelium to wall off the periodontal tissues from the bacterial biofilm, before the involvement of chronic inflammatory cells (Miyasaki, 1991). An increased quantity of PMNs has also been noted to occur in the sulci of chronically inflamed gingiva (Attstrom, 1970). The protection conferred by the host response effectively defends against the bacterial insults that constantly threaten the health status of the periodontium in the majority of the population (Deas, Mackey, & McDonnell, 2003; Schenkein, 2006).

**The Neutrophil’s Protective and Destructive Mechanisms**

Although the PMN is a protective cell, it can by way of its inherent activity, also contribute to destruction of the local tissues that it has infiltrated to defend against bacterial invasion. The PMN employs two systems for bacterial killing, which are distinguished by their dependence on oxygen. The oxygen-independent system or “degranulation”, was described earlier, and releases enzymes through degranulation of primary and secondary granules in the cell cytoplasm. The oxygen-dependent pathway is further divided into two types. The myeloperoxidase-independent pathway results in free radical generation where nicotinamide
adenine dinucleotide phosphate oxidase (NADPH) is bound to the cell membrane, and catalyzes the reduction of oxygen to superoxide anion, which is converted to hydrogen peroxide and hydroxyl radical. These free radical metabolites are only toxic to pathogens at high concentrations. The myeloperoxidase-dependent pathway involves the reaction between hydrogen peroxide and halide to form hypochlorous acid, chlorine, and the hypochlorite ion, which is highly toxic to bacteria, fungi, viruses, and mycoplasma, due to its intense oxidative capacity (Van Dyke & Hoop, 1990; Lamster, 1992; Trowbridge & Emling, 1997; Deas, Mackey, & McDonnell, 2003).

However as alluded to above, the protection provided by the PMN is not without consequence. As noted previously, the PMN plays a major role in the host response, and normally plays a protective role, defending against bacterial insults that threaten gingival health (Deas, Mackey, & McDonnell, 2003). Yet, these killing mechanisms have the potential to damage extracellular tissues via the release of granular constituents such as collagenases and MPOs, and reactive oxygen species (Van Dyke & Hoop, 1990; Lamster, 1992; Deas, Mackey, & McDonnell, 2003). In addition, ineffective or excessive response by the host can lead to tissue damage – an outcome that is influenced by individual factors (Miyasaki, 1991; Hart, Shapira, & Van Dyke, 1994; Van Dyke & Serhan, 2003). The destruction incurred may be dependent on the amount of time that the PMN is allowed to be present in the tissues. Persistently present PMNs, as in the case of gingivitis, where bacteria are consistently present can lead to tissue damage (Nussbaum & Shapira, 2011). Cell death through apoptosis as opposed to necrosis is critical in order to preserve tissue function and integrity. Apoptotic cells help to resolve inflammation by sending signals to macrophages,
which engulf the PMNs and secrete anti-inflammatory cytokines, such as transforming growth factor beta (Fadok & Henson, 1998). If apoptosis is reduced, as is thought to occur in periodontal disease, then the macrophages that help to regulate the inflammatory response will also decrease, and the PMNs that are present will continue to release their destructive contents (Nussbaum & Shapira, 2011). Thus, in addition to killing bacteria, these cells continue to release products that can cause direct damage to extracellular matrices as well as other host cells.

**A Focus on Bacteremias**

Periodontal infections are characterized by recurrent bacteremias, as evidenced by the identification of oral pathogens systemically (Offenbacher & Beck, 2005). The chronic and recurrent nature of periodontal disease allows for repeated hematogenous dissemination of periodontal microorganisms and exposure of the vasculature to oral microbes (Beck & Offenbacher, 2002). The microflora that reside in the oral biofilm are able to endure the hostile oral environment which is constantly under attack by host defenses such as serum antibody, complement, leukocytes, and salivary proteins. In the presence of inflammation, these organisms are able to thrive, invade host tissues, release factors such as lipopolysaccharides, and enter the systemic circulation (Offenbacher, Elter, Lin, & Beck, 2005). This can trigger the production of several systemic pro-inflammatory cytokines and mediators of tissue destruction such as C-reactive protein, TNFa, prostaglandin E2, and other cytokines (Kuo, Polson, & Kang, 2008). Patients with poor oral hygiene are at greater risk for the development of bacteremia, which increases with the severity of gingival
inflammation (Silver, Martin, & McBride, 1977; de Oliveira, Watt, & Hamer, 2010). As will be discussed in more detail below, this is especially important in patients with special needs.

**A Focus on Systemic Inflammation**

C reactive protein (CRP) and fibrinogen can be used as markers to evaluate the inflammatory status of an individual, and could be useful predictors of future cardiovascular events in various populations (Danesh et al., 2000). An association between periodontitis and increased CRP levels was first demonstrated in 1997 (Ebersole, Machen, Steffen & Willmann, 1997). More recent reports suggest that non-surgical periodontal treatment such as scaling and root planing can reduce CRP levels significantly (D’Aiuto, Ready, & Tonetti, 2004; Lopez et al., 2011; Siribamrungrong & Puangpanngam, 2012). Notably, patients who brush their teeth infrequently (i.e. those with poor oral hygiene), had increased concentrations of CRP and fibrinogen when compared to those with good oral hygiene levels (de Oliveira, Watt, & Hamer, 2010). Furthermore, periodontal disease has been associated with moderate systemic inflammatory responses (D’Aiuto, Ready, & Tonetti, 2004), as shown by elevated levels of CRP and other inflammatory biomarkers (Loos, Craandijk, Hoek, Wertheim-van Dillen, & van der Velden, 2000). Thus, a higher individual inflammatory load may result in increased cardiovascular risk based on serum CRP and fibrinogen concentrations due to the systemic inflammatory response consequent to low grade chronic periodontal infections (D’Aiuto, Ready, & Tonetti, 2004). Hence, in addition to the putative problems caused by bacteremia, these findings are noteworthy as periodontal disease is a condition that presents with modifiable risk factors (inflammation and infection),
which can be prevented and treated with predictable treatments that have minimal risk, as will be discussed later.

**Oral Inflammation and Respiratory Tract Infections**

The evidence demonstrating an association between aspiration of pathogenic oral microorganisms and chronic lung infections is increasing (Scannapieco, Papandonatos, & Dunford, 1998; Scannapieco, 1999; Azarpazhooh & Leake, 2006; Paju & Scannapieco, 2007; Raghavendran, Mylotte, & Scannapieco, 2007). In addition to direct microbial induction of aspirational respiratory infections, there is also evidence that host-derived mediators such as inflammatory cytokines, which are found in high levels in the saliva of patients with periodontal disease, could induce lung inflammation when aspirated and can lead to conditions such as pneumonia and chronic obstructive pulmonary disease (Scannapieco, 1999). Aspiration of bacteria from the oral cavity is a common route of infection for bacterial pneumonia, with dental plaque being a source of potential infectious agents and periodontal pockets acting as reservoirs (Azarpazhooh & Leake, 2006; Paju et al., 2009). Scannapeico (1999) noted four possible mechanisms whereby oral bacteria may exert their pathogenic role. First, pulmonary pathogens may colonize dental plaque which is then aspirated. Second, bacterial enzymes associated with the pathogenesis of periodontal disease may assist respiratory pathogens in adhering to the airway. Third, hydrolytic enzymes in periodontitis may destroy the protective salivary pellicle on pathogenic bacteria, thus reducing bacterial clearance from mucosal surfaces via host protective mechanisms. Fourth, the cytokines released by inflammed periodontal tissues and peripheral cells may cause increased adherence receptors to be expressed on the mucosal surface and allow for
respiratory pathogen colonization. Other proposed mechanisms for pulmonary infection are inhalation of air-borne bacteria or bacterial translocation by bacteremias (Paju & Scannapieco, 2007). Poor oral hygiene has been associated with a significant increase in likelihood of chronic respiratory disease and aspiration pneumonia in susceptible individuals, such as patients in intensive care units and notably for the purposes of this investigation, in patients with special needs (Scannapieco, 2005b; Sigal & Sigal, 2006). This is something that should be highly modifiable and in fact in 2006, Azarpazhooh and Leake reported that there is fair evidence demonstrating an association between oral status and pneumonia, and good evidence that improving oral hygiene and frequent professional oral health practices decreases the occurrence or progression of respiratory diseases. However, there might not be a simple approach to this issue insofar as patients with special needs are concerned.

The Special Needs Population at Increased Risk for Disease

The literature shows that patients with special needs have more plaque and lower levels of oral hygiene than the general population (Anders & Davis, 2010). Patients with special needs often present with poor oral hygiene or are unable to maintain oral hygiene due to motor skill impairment, lack of self-help skills and understanding of complex tasks, poor cooperation, manual dexterity and coordination, or ineffective technique (Lindemann, Zaschel-Grob, Opp, Lewis, & Lewis, 2001). The importance of maintaining a level of optimal oral health in patients with special needs is clarified when recognizing the decreased ability of the body to defend itself in those with reduced salivary flow, diminished cough reflex, swallowing disorders, inability to perform or maintain good oral hygiene, or other physical disabilities (Thornton, al-Zahid, Campbell, Marchetti, & Bradley, 1989; Teng et al.,
Poor oral hygiene can be even worse in situations where the oral musculature is impaired so that natural cleansing of the oral cavity is limited (Shaw, Shaw, & Foster, 1989). In addition, patients who are not able to cooperate for dental treatment have been shown to lose more teeth secondary to periodontal disease or caries than patients who are cooperative (Gabre, Martinsson, & Gahnberg, 1999).

In most cases, the provision of oral hygiene is dependent on the caregiver. Therefore, the caregiver must be knowledgeable and proficient with regard to the delivery of oral care for their charges, and must also have an understanding of the importance to the patient of having a clean mouth (Cumella, Ransford, Lyons, & Burnham, 2000; Christensen, 2005). Unfortunately, most caregivers do not receive formal training in oral hygiene regimes (Cumella, Ransford, Lyons, & Burnham, 2000), and thus the patients under their care do not have optimal oral hygiene or oral health in many cases.

In the face of poor oral hygiene, development of an anaerobic environment below the gingival margin ensues leading to the formation of subgingival plaque (i.e. a pathogenic biofilm). Consequently, at this point, professional dental care is required for complete removal of plaque. Although all forms of gingival inflammation do not necessarily develop into inflammatory periodontal disease, the development of this type of inflammation is still considered as one of the prerequisites for this tissue-destructive condition to develop (Boehm & Scannapieco, 2007). The potential role of bacteria contained within the oral biofilms as contributors to respiratory infections has already been discussed but it bears repeating that by improving oral hygiene and establishing a level of optimal oral health, this could also lead to improved periodontal health (Teng et al., 2002; Binkley, Haugh, Kitchens, Wallace, &
Sessler, 2009), and perhaps an improvement in the overall morbidity and mortality rates seen in this population of patients (Sigal & Sigal, 2006). If this was the only problem to consider it would still represent a formidable concern. However, as will be discussed in more detail below, other issues that interfere with the establishment of optimal oral health include a general inability to establish diagnoses of oral disease and in concert, difficulties and in some cases inability to treat oral disease once identified in the special needs population (unless the patient can be placed under a general anaesthetic).

**Treatment of Periodontal Diseases**

The primary goal of any therapeutic intervention in inflammatory disease is to restore the tissue to a state of homeostasis. Treatment for periodontal diseases aims to change or eliminate the causative organisms to decrease or eliminate inflammation, establish periodontal health, arrest disease progression, and prevent recurrence (Kim & Amar, 2006; Van Dyke, 2007). It follows that regular disruption of the biofilm via regular oral hygiene practices allows for maintenance of a biofilm that is compatible with periodontal health, and personal and professional care is integral to preventing re-initiation of inflammation (Sanz & van Winkelhoff, 2011). Treatment objectives are complicated where an acute inflammatory response has progressed to chronic inflammation, as evidenced by tissue damage of the cellular matrix, scarring, fibrosis, or features of inadequate healing. Thus, timely clinical diagnosis and appropriate treatment intervention and host response (healing) is required to prevent the progression of disease from acute to chronic disease, for it to be more likely to achieve homeostasis (Van Dyke, 2007).
The foundation of treatment for periodontitis initially involves non-surgical debridement, referred to as scaling and root planing. This can be performed with manual instruments or electronic devices and the goal is to remove dental plaque and calculus, thereby reducing, eliminating, or at least controlling the pathogenic oral biofilm. When combined with regular personal oral hygiene, tissue inflammation is reduced and clinical attachment can be improved (Cobb, 1996; Drisko, 2001; Cobb, 2002; Suvan, 2005). A recent study has shown that non-surgical periodontal therapy also improves oral health-related quality of life (Wong, Ng, Corbet, & Keung Leung, 2012). If unsuccessful, non-surgical periodontal therapy may be supplemented with anti-bacterial treatment, or if that is not successful access surgery may be required (Kim & Amar, 2006). The patient’s response to treatment is monitored at follow-up examinations at regular intervals, depending on the extent of disease and ability to maintain oral hygiene (Cohen, 2003; Pihlstrom, Michalowicz, & Johnson, 2005). Treatment is individualized at these appointments and typically consists of debridement, oral hygiene instructions, and continuation of attempts to control or eliminate risk factors for disease (Lamster, 1996; Renvert & Persson, 2004).

The anti-microbial approach to periodontal therapy is aimed at reducing the inflammatory load at the tooth and gingival interface. As previously noted, a consistent oral hygiene regimen consisting of tooth brushing and flossing to disrupt the oral biofilm is the mainstay in periodontal treatment. Despite attempts to manage the oral biofilm with such mechanical measures by the dentist and patient, clinical progression and signs of disease activity may persist, and therefore supplementation with chemotherapeutic agents is often considered to aid in the achievement of periodontal health. Chemotherapeutic agents include antiseptics
that are applied topically, but do not contain antibiotics or disinfectants, and therefore do not provide a risk for bacterial resistance. Oral rinses such as chlorhexidine gluconate (Peridex™), irrigants such as hydrogen peroxide, and triclosan-containing toothpastes are examples of agents that are included in this category. Host modulation therapy is a newer approach to managing disease by inhibiting mediators of host tissue destruction such as MMPs, cytokines, and prostanoids. These agents include topical and systemic non-steroidal anti-inflammatory drugs, subantimicrobial-dose doxycycline, and systemic bisphosphonates (Ryan, 2005). The application of non-surgical therapy in the management of periodontal disease relies on proper case selection and compliance, including frequent re-evaluation and monitoring in order to achieve long term success (Drisko, 2001).

Conventional Methods for Diagnosis of Periodontal Diseases

The Periodontal Probe

The periodontal probe is the standard tool of measure used to distinguish between gingivitis and periodontitis (Ranney, 1993), and is based on readings of periodontal probing depths and clinical attachment loss (Armitage, 1999). However, problems with the conventional periodontal probe in its application to the clinical setting have been noted, even for patients who are not in the special needs category. For example, penetration of the probe is influenced by inflammation of the tissues, so that the probe tip can extend beyond the apical termination of the junctional epithelium during measurements compared to the healthy state (Anderson, Caffesse, Nasjleti, & Smith, 1991). Reproducing measurements using the periodontal probe is also challenging due to difficulties in replicating position, angulation, and insertion force (Khan & Cabanilla, 2009). Probing also has a notable measurement error.
of ± 1 mm. Objectively, this associates a significant measuring error to a pocket depth measurement, where a 5 mm probing depth would be associated with a 40% (2/5 mm) error. The clinical implications of this are important to consider when assessing the diagnostic value of the periodontal probe and whether measurements using this instrument can be used reliably to determine whether the periodontal lesion in periodontitis is active or not. If the associated errors are not considered during interpretation, a patient could be identified incorrectly as being healthy or diseased due to measuring error, which could result in inappropriate treatment recommendations (Apsey, Kaciroti, & Loesche, 2006). Because the probing error is so large, it might be possible to consider probing depth measurements as being non-parametric, indicating that mean probing values should also probably be avoided in preference to frequency distributions. Finally, the periodontal probe demonstrates disease after active disease is already present and has caused damage to the periodontium (Lamster, Celenti, Jans, Fine, & Grbic, 1993), and cannot be used to prognosticate additional tissue destruction or, as alluded to above, to differentiate between an ‘active’ periodontal lesion and a quiescent (i.e. non-progressing) lesion.

Clinical Indices
Clinical parameters that are currently used for the assessment of gingival health status include: erythema, edema due to swelling itself or possibly due to hyperplasia of underlying connective tissues, accumulation of bacterial plaque and calculus, increased probing depths (i.e. above any beyond the probing depths accepted as representing normalcy; 0-3 mm), bleeding on probing, clinical attachment loss, gingival recession, furcation involvement, tooth mobility, and radiographic evidence for loss of alveolar bone (Haffajee, Socransky, &
Goodson, 1983; Mariotti, 1999; Pihlstrom, 2001). However, these parameters have also been noted to have numerous limitations, in that they only provide measures of past disease activity, and cannot be used to determine current disease activity or to prognosticate as to progression of disease and tissue destruction in the future (Haffajee et al., 1991; Armitage, 1996).

Numerous indexing systems have been created to grade the severity and extent of gingival and periodontal diseases, in order to translate the above clinical findings into numerically scaled quantities. Indices such as the periodontal disease index (Ramfjord, 1959), and the oral hygiene index (Greene & Vermillion, 1964), were developed to serve as tools for rapid screening of large populations for periodontal disease (Ainamo & Bay, 1975). This has allowed for determination of the prevalence of gingival diseases in large populations, as well as evaluation of treatment outcomes. Specific teeth or the entire dentition are measured at 4 or 6 sites and may then be averaged to specify the mean score for the entire mouth. Thus, the indices provide semi-quantitative information about disease severity and can be analyzed statistically (Ramfjord, 1959). Unfortunately, when it comes to the analysis of discontinuous or non-parametric scales, the use of mean data may not allow for robust statistical analysis. The pitfall in using mean individual scores is that the implication of disease when relating scores from 0 to 1 compared to 1 to 2 are not equivalent (Ainamo & Bay, 1975). Furthermore, an ordinal score of ‘3’ is not three times worse than an ordinal score of ‘1’, which further underscores the inappropriateness of using mean data when applying the various indices for assessment and quantification of periodontal diseases. Moreover, there is a degree of subjectivity involved in the use of periodontal disease indices including the
presence of measurement error due to inter- and intra-examiner differences (Haffajee et al., 1991; Lamster, Celenti, Jans, Fine, & Grbic, 1993; Pihlstrom, 2001). It is also noteworthy that different categories within the different indices are divided and detailed in order to be more sensitive to variation, but can result in problems with examiner reliability, where the examiner has difficulty in assigning a score – and assigning the score consistently – when there are more categories available (Greene, 1967; Benamghar, Penaud, Kaminsky, Abt, & Martin, 1982; Kingman, Löe, Anerud, & Boysen, 1991). Finally, these scales lack the specificity and objectivity needed for evaluation of an individual patient’s disease status (Klinkhamer, 1968).

Limitations in Diagnosis of Periodontal Diseases

Diagnosis of gingival inflammation in the clinical setting is limited to traditional methods of assessing disease that have now been described. Conventional measures of periodontal disease can be divided into two main approaches: those used to assess severity of disease, or those concerned with activity of disease. Severity of disease can be defined as the periodontal health status at the time of examination, and is measured using the various measures noted above. In contrast, measurement of disease activity (e.g. progression of periodontal attachment loss), is more difficult as it relies on changes in the clinical parameters used to assess periodontal disease over a specific period of time (Lamster, Celenti, Jans, Fine, & Grbic, 1993); parameters which themselves are open to significant variation and in and of themselves, given a particular single time of assessment, do not necessarily convey whether disease is active or not. In this regard, disease activity is measured once damage to the periodontium has already occurred (e.g. progressive
attachment loss; Lang, Joss, Orsanic, Gusberti, & Siegrist, 1986). This type of measurement cannot be used for reliable prognostication of future disease activity. Diagnosis is complicated further by studies showing that periodontal diseases do not appear to follow a linear pattern of progression, but rather are characterized by periods of latency and exacerbations (Goodson, Tanner, Haffajee, & Sornberger, Socransky, 1982; Socransky, Haffajee, Goodson, & Lindhe, 1984). Therefore, even in cases where severe destruction of tissue has been demonstrated, this information cannot be used unequivocally to predict that future disease progression will ensue. Indeed, it has been shown in an assessment of the clinical parameters for periodontitis that are currently used in dental practice, that none were useful in predicting disease activity at individual sites when used either individually or in combination (Haffajee, Socransky, & Goodson, 1983).

**Limitations of Diagnosis in the Special Needs Population**

The measurement of the above clinical parameters in the uncooperative special needs population with accuracy, efficiency, and safety is a challenge. In addition to the problems associated with measurement of periodontal disease severity and activity in otherwise cooperative patients, it cannot be overstated that most methods of measurement in the uncooperative special needs population are even more difficult and in some cases virtually impossible. Studies that attempt to assess the oral health status of patients with disabilities are unable to compare the periodontal conditions adequately due to difficulties in acquiring periodontal indices or measures that require patient cooperation, which could actually be traumatic if attempted. Therefore, any measurements made are suspect insofar as accuracy is concerned. In this regard, a conventional periodontal examination with visual inspection and
a periodontal probe may be impossible due to lack of patient cooperation (Hennequin, Faulks, & Roux, 2000). This means that we not only have little information regarding the true periodontal status of such patients (until they are placed under a general anaesthetic), but any efforts to develop novel and more user-friendly treatments for patients in this population are substantially hampered due to the inability to follow the progression or regression of periodontitis without placing such patients under a general anaesthetic more often than would be appropriate from a clinical and ethical standpoint. Unfortunately, literature to propose or support the use of alternative strategies that could be used for diagnosis of gingival or periodontal health status in the special needs population is lacking. In fact, oral health needs are often underestimated by caregivers and dental health professionals due to the patients’ inability to express pain or discomfort, and an inability to obtain reliable clinical data using any number of diagnostic tests that, in a cooperative population could be done, but cannot be done in the uncooperative population of special needs patients (e.g. vitality testing, periodontal probing). As already stated above, the limitations in diagnostic tests available to assess the periodontal status of the uncooperative special needs patient in a non-invasive manner precludes accurate diagnosis and prognostication. Therefore, it is also not possible to formulate individualized and appropriate treatment plans designed to place such patients in a position of optimal oral health, or to monitor the outcomes of treatment accurately.

**The Visual Analog Scale as a Measure of Gingival Inflammation**

The Visual Analog Scale (VAS) is a measurement that is often used for the subjective assessment of dental pain (Seymour, Charlton, & Phillips, 1983), and has also been noted as an effective means of evaluation for patient perceptions of post-operative variables after
periodontal treatment (Matthews & McCulloch, 1993). Notably, its application has been suggested for use by the patient as a quantitative yet subjective indicator in order to guide the clinicians’ assessment. However, the literature does not include the VAS as a periodontal measure for inflammation, and indices such as the gingival bleeding index (Ainamo & Bay, 1975), rather than the VAS, are traditionally used during periodontal examination as discussed earlier. In the uncooperative special needs population, the time available for patient assessment is limited due to the potential for rapid deterioration of behaviour with longer appointment times. In this case, measurement of various indices that involve placing the clinical picture within multiple categories by the examiner can be time-consuming and impractical. Thus, the current assessment of periodontal health in this population often involves an overall impression or use of a VAS, regarding severity and prognosis of periodontal disease and consequent treatment needs.

**Novel Methods for Assessment of Periodontal Disease Activity**

An understanding of the limitations of the currently used clinical measures of periodontal health status seriously underscores the necessity for pursuit of other and better diagnostics for periodontal disease (Apsey, Kaciroti, & Loesche, 2006). Ideally, a clinical parameter could be used to monitor periodontal destruction in three ways. It could record active disease, allow for quantitative monitoring of treatment responses, and indicate susceptibility to disease (Fine & Mandel, 1986). Diagnostic tests for periodontal disease are useful in patients that have not received periodontal therapy, providing a baseline measure of disease status. Following periodontal treatment, diagnostic tests may demonstrate responses to treatment and identify disease progression and future risk of disease, which is directly helpful in the
clinical situation in directing subsequent treatment, including determination of need and extent of future treatment (Lamster, Celenti, Jans, Fine, & Grbic, 1993).

In light of the limitations noted with the current methods used for the diagnosis and assessment of periodontal disease severity, there have been efforts to develop diagnostic tests that are based on evaluation of factors that are thought to play an important role in the pathogenesis of periodontal diseases, with emphasis not only on its presence or severity, but also the activity of the disease once identified. Wherever possible the goals have been to develop diagnostic tools that are as objective and quantitative as possible which would allow for the identification of the different presentations of periodontal disease, measurement of disease activity and progression, while also allowing for the prediction of future disease activity (Fine & Mandel, 1986; Cao & Smith, 1989; Lamster, Celenti, Jans, Fine, & Grbic, 1993; Ranney, 1993). Current supplemental diagnostic tests are focused on different aspects of the disease that have been identified to play important roles in the pathophysiological processes of periodontitis including: detection of microbial periodontal pathogens, assessment of host-derived enzymes that might degrade periodontal tissues, identification of breakdown products from diseased periodontium, mediators of inflammation, and genetic testing (the latter predominantly for assessment of disease-risk). Although these tests have provided valuable information, they cannot be applied readily to the clinical setting as they require increased clinical time to be performed, while they also require specialized equipment and training to be used properly (Fine & Mandel, 1986; Landzberg, Yuen, & Glogauer, 2008).
Gingival Crevicular Fluid – Insights and Limitations

Investigation of the constituents of gingival crevicular fluid (GCF) has been an active area of research focusing on the pathophysiology of periodontal disease. Constituents of GCF are derived from many sources, including substances from the host as well as from the microorganisms inhabiting the subgingival biofilm. The contents of GCF mirror the makeup of serum (being in large part a serum transudate), while GCF also contains the cytokines and products of periodontal metabolism noted above (as inflammatory exudate; Lamster & Ahlo, 2007). More than 65 components of GCF have been tested as potential diagnostic markers for periodontal disease progression, and have been grouped into three categories: 1) host-derived enzymes and their inhibitors, 2) inflammatory mediators and host-response modifiers, and 3) byproducts of tissue breakdown (Armitage, 2004). In understanding the constituents of GCF it has been hoped that changes in any one of its several components might be used to demonstrate the presence, severity, progression or likelihood of progression of periodontal disease in a given area.

Analysis of GCF seems to have the potential to contribute to the development of valuable diagnostic tests, but its application is still limited in the dental practice setting. With respect to ease of use, it is a relatively simple task to collect GCF from the gingival sulcus surrounding the teeth for later assessment. Saliva can be collected non-invasively by persons who have limited training, and special equipment for collection of saliva is typically not required. Saliva collection is also well tolerated by patients and may be cost-effective when screening large populations (Kaufman & Lamster, 2002). However, collection of GCF requires some time and multiple samples are often taken from each patient. Also, selection
of the tooth or teeth to be used for collection of GCF can be subjective or random meaning that identification of sites that are at-risk for further breakdown, or are at the very least ‘diseased’ can be difficult (Lamster & Ahlo, 2007). Clinicians may also lack comfort with using some of the diagnostic tests currently under development, particularly when the tests might be used for the assessment of health status and formulation of ongoing treatment plans. As well, it is not clear that the currently available tests are accurate enough to provide reliable information regarding disease activity and progression, and so the potential benefit to patients is not clear (Kaufman & Lamster, 2000). Hence, the diagnostic value of these tests has not been sufficient enough to be incorporated widely into the dental practice setting, nor are they easy enough to be used as a routine chair-side test. Moreover, it is also important to point out that performing tests that rely on assessment of GCF are simply not possible on uncooperative patients with special needs.

**Evaluation of Oral Neutrophils in Periodontal Inflammation**

The orogranulocytic migratory rate (OMR) index was developed by Klinkhamer (1968), as an objective method for quantifying one aspect of the inflammatory process in the periodontium. This test is based predominantly on the rate of leukocyte migration from the gingival sulcus to the oral cavity. It was proposed that the increase in vascularity and cell migration, as in cases of periodontal inflammation, could be measured by the OMR index. When an inflammatory reaction occurs in periodontal tissues, the rate of cell migration is increased, leading to increased formation of cellular infiltrate and increased granulocyte migration (Klinkhamer & Zimmerman, 1969). Woolweaver, Koch, Crawford, and Lundblad (1972) demonstrated that the predominant leukocyte in oral saline rinses was the PMN,
which appeared to retain its vitality and function. The OMR was also shown to increase in relation to increased gingival inflammation, and also correlated with increased pocket depths (Klinkhamer & Zimmerman, 1969). Hence, the OMR index was proposed as a reliable indicator of the severity of periodontal disease, and most importantly, it did not require ‘clinical judgment’ for interpretation (Klinkhamer, 1968). However, the OMR index was determined by counting the number of oral granulocytes present in a series of twelve sequential oral mouthwashes, lasting 30 seconds each, making it difficult to apply to the clinical setting. Again, this is rather time-consuming and difficult for cooperative patients and when it comes to uncooperative special needs patients, it is virtually impossible to carry out reliably and accurately.

**Oral Neutrophil Quantification to Assess Periodontal Disease Status**

Recognition that the quantity of crevicular leukocytes could be used as a potential indicator for gingival inflammation was described in 1970 (Attstrom, 1970). It was first proposed in 1978 to quantify oral PMN levels in order to assess the status of periodontal disease as well as responses to treatment in patients (Raeste & Aura, 1978). As also discussed above, it has been shown repeatedly that the quantity of oral PMNs varies directly with the extent of gingival inflammation (Attstrom, 1970; Schiött & Löe, 1970). In addition, oral PMN counts correlate positively with increasing pocket depths and the gingival index (Woolweaver, Koch, Crawford, & Lundblad, 1972). In developing a simple method for oral neutrophil quantification, a non-invasive oral rinse assay modified from Wright, Meierovics, & Foxley (1986), was validated at the University of Toronto’s Department of Periodontology in 2006. Using this approach, patients with chronic periodontal disease provided oral rinses before
and after phase I periodontal treatment. Samples were then processed in the lab and PMNs were counted, and it was found that following initial treatment, there were clear reductions in the levels of oral PMNs. Given this finding, it was anticipated that such an assay could be used to monitor disease activity, severity, and progression (Bender, Thang, & Glogauer, 2006).

The oral rinse assay has also been used successfully in the medical field to monitor PMN delivery in extracellular tissues (Wright, Meierovics, & Foxley, 1986; Akpek, Knight, & Wright, 2003; Cheretakis, Dror, & Glogauer, 2005). Wright, Meierovics, and Foxley (1986) have shown that acute neutropenia following chemotherapy translated to reduced oral PMN levels. Investigators have also demonstrated a direct correlation between oral and blood PMNs in adults undergoing myelosuppressive chemotherapy (Akpek, Knight, & Wright, 2003). Cheretakis, Dror, and Glogauer (2005) observed PMN recovery in myeloid engraftment haematopoietic stem cell transplantation. It is important to note that the changes in oral PMN levels in both studies occurred prior to detection of changes in blood PMNs (Wright, Meierovics, & Foxley, 1986; Akpek, Knight, & Wright, 2003). Thus, it has been suggested that the presence of PMNs in tissues could be a better indicator of susceptibility to infection than circulating white blood cell counts, and might actually predict marrow engraftment better than tests of peripheral levels of PMNs (Wright, Meierovics, & Foxley, 1986).
Limitations in Diagnosis with the Oral Rinse Assay

Advantages of the oral rinse assay include large volumes available for analyses and ease of sample collection. However, the oral rinse assay cannot be applied to the clinical setting yet, due to the reliance on lab-based techniques (i.e. PMN counting using the microscope). In addition, salivary constituents arise from many sources, including serum and host factors in GCF such as epithelial cells, inflammatory cells, cell mediators, subgingival and supragingival bacteria, oral epithelial cells that have sloughed, and foreign substances such as food and oral hygiene products (Lamster & Grbic, 1995). Moreover, cells that are collected in the salivary samples are non-specific, and include cells from the entire oral cavity, which could be influenced by the presence of other infective or inflammatory lesions involving the oral mucosa. The oral rinse also dilutes the cells that are acquired from the main area of interest – the gingival sulcus. Most importantly for the purposes of this investigation, the use of the oral rinse assay is impractical if not outright impossible in patients who lack the cooperation and/or coordination required to rinse and expectorate reliably.

Relation of Myeloperoxidase to Periodontal Disease

Myeloperoxidases (MPO) are neutrophil specific enzymes that are involved in PMN bacterial killing mechanisms via the oxidation of chlorine ion as described earlier. MPO is a heme containing protein and the unique ability of this enzyme to oxidize chlorine is recognized to be vital for the host defense system by killing invading pathogens (Gaut et al., 2001). Thus, MPOs act as a specific marker of primary PMN granules released in response to offending stimuli (Gessler, Pfenninger, Pfammatter, Carrel, & Dahinden, 2002). MPO measurement has shown to be a good model for estimating PMN quantities in inflamed tissues (Bradley,
MPO has been found at higher levels in patients with periodontal disease (Smith, Hinrichs, & Melnyk, 1986), and at varying concentrations that correlates with the clinical severity of periodontitis (Wolff et al., 1988). Studies have also shown an increased level of activity and quantity of MPOs at the sulcus as a result of inflammation at periodontitis sites (Cao & Smith, 1989; Gomes et al., 2009). Decreased MPO activity has also been demonstrated following periodontal treatment (Smith, Hinrichs, & Melnyk, 1986; Wolff et al., 1988). MPO measurement offers a simple and less technical procedure for estimating PMN presence in the gingival crevice compared to PMN counting (Cao & Smith, 1989). Since the quantity of PMNs can be taken as a measure of the intensity of the disease process, MPOs can be used as a marker of PMN content, and would prove useful in both the diagnosis and evaluation of treatment responses geared towards resolving inflammatory conditions (Bradley, Priebat, Christensen, & Rothstein, 1982). Animal models have revealed PMN MPO as a good indicator of systemic rather than local inflammation in a chronic inflammatory condition (Queiroz-Junior et al., 2009). Furthermore, measurement of MPO has already been applied in the medical field successfully to monitor PMN activity after cardiopulmonary bypass in paediatric cardiac patients (Gessler, Pfenninger, Pfammatter, Carrel, & Dahinden, 2002). Though MPOs are not a specific marker of periodontitis, it is postulated that increased levels of MPOs from the GCF may reflect the number of PMNs present in the gingival sulcus, and further may reveal the extent of gingival inflammation (Cao & Smith, 1989).
Oral Neutrophil Quantification – A Novel Approach

Recognition of the aforementioned limitations has led to the development of a novel technique that quantifies PMNs, using an oral swab or Q-tip™, which is traced along the buccal surfaces of the upper and lower dental arches, along the tooth crown and gingival interface. GCF is absorbed onto the swab head and the swab is then dipped in sterile saline to make a solution containing the lysed PMNs and their contents, including MPO. This technique uses a colourimetric reaction to measure oral PMNs present on the swab via MPO activity. Hydrogen peroxide is added to each PMN-containing solution in order to activate a chromogenic reagent called 2, 2’ – azinobis (3-ethylbenzothiazoline-6-sulphonic acid), also known as ABTS. In turn, ABTS contains diammonium salts that react with MPOs present in PMN granules and this reaction leads to a visual colour change (blue). The intensity or optical density of the blue stain (i.e. the darkness of the blue color), correlates with increased numbers of PMNs in the sample (Landzberg, 2009). Previous tests have confirmed that the colourimetric reaction does not occur with a control or sterile swab alone (M. Glogauer, personal communication, July 7, 2010). The colourimetric reaction is measured by comparing the resulting blue sample colour change by eye, mechanically to a standard curve produced using blood neutrophils, or using ultraviolet light to measure the sample’s absorbance (with the FLUOstar Optima microplate reader). The PMN assay using the oral swab technique has been validated for accuracy and reproducibility, and correlates well to the PMN counts derived from the oral rinse technique (M. Glogauer, personal communication, July 7, 2010). Thus, oral PMNs in the sample can be quantified to provide an objective measure of gingival and periodontal inflammation, thereby providing valuable diagnostic information about gingival health status, and may identify early signs of inflammation that
may lead to future tissue breakdown. The PMN assay may also provide a standardized method for clinical measurement of periodontal inflammation in patients who cannot be examined using routine methods; that is, the uncooperative special needs patient population. This would help to overcome the problem of subjectivity met with conventional measures of periodontal disease in all populations, and particularly in the special needs population, and would be extremely helpful insofar as assessment of treatment outcomes are concerned.

**RESEARCH OBJECTIVES**

- To assess the baseline gingival health status of uncooperative patients with special needs attending Toronto’s Mount Sinai Hospital Dental Program for Persons with Disabilities using the PMN assay and conventional methods
- To validate the PMN assay by correlating levels of gingival inflammation as assessed by PMN counts collected from the oral swab technique with conventional clinical parameters in patients with special needs receiving comprehensive dental care under GA, and at subsequent recall examination
- To monitor responses to periodontal treatment by comparing gingival inflammation as assessed by the PMN assay and conventional methods pre-treatment under GA and at the subsequent follow-up examination
- To assess the usefulness and feasibility of the PMN assay as a quantitative measure of periodontal inflammatory disease in the uncooperative special needs patient population
HYPOTHESES

1. All of the uncooperative patients with special needs who are assessed in this cohort will demonstrate a high prevalence of gingival inflammation, regardless of what diagnostic methods are used.

2. Oral PMN levels derived from oral swabs will correlate with traditional measures of gingival inflammation.

3. The PMN assay will allow for monitoring of gingival health status and responses to treatment when comparing pre- and post- treatment evaluations.

4. It will be feasible to use the PMN assay using the oral swab technique to assess gingival inflammation in the uncooperative special needs population.
STUDY DESIGN AND METHODS

Ethics Review

Prior to study commencement, the research protocol for this project was approved by the Mount Sinai Hospital Research Ethics Board and the Office of Research Ethics at the University of Toronto, Toronto, Ontario, Canada.

Sample Size Calculation

Sample size calculations were performed prior to study commencement using sample size calculation software (Faul, Erdfelder, Lang, & Buchner, 2007). The required sample size to detect a correlation of at least 0.4 ($\rho_{\text{smallest}}$) or greater between changes in probing depths and neutrophil counts at an $\alpha = 0.05$ significance level, with 80% power ($1-\beta = 0.80$), using a two-sided test ($H_0: \rho = 0$ versus $H_1: \rho \neq 0$), was calculated as 44 patients.

Funding

Funding for this research project was provided by the Department of Dentistry at Mount Sinai Hospital, Toronto, Ontario, Canada.

Conflict of Interest

The investigators of this study have no conflicts of interest. The dental care and treatment of the patients involved was not affected in any way, regardless of their participation. The investigators did not receive any sponsorship.
Examiner Training and Calibration

A multi-examiner approach was inherent to this study due to the operating room and staffing schedules at the Mount Sinai Hospital Dental Clinic. The evaluators in this study consisted of Mount Sinai Hospital General Dental Residents and University of Toronto Paediatric Dental Graduate Residents. The oral swab and periodontal examination data that was collected in the operating room and on the follow-up examination was performed by residents who were trained according to the definitions and format specific to this study, to ensure standardization.

Evaluator training consisted of a calibration exercise, which consisted of 2 separate evening sessions. In the first session, the residents attended a thirty minute presentation given by the trainer (A.M.), which provided an overview of the research study, reviewed the study protocol, and the definitions and techniques involved in data collection. Each evaluator then demonstrated the oral swab technique intra-orally on another evaluator, while being observed by the trainer to ensure proper technique. The oral swabs (MedPro Sterile Cotton Tipped Applicators 6”, AMG Medical Inc., Montreal, Canada) were then taken to the lab for oral PMN quantification. Next, each evaluator performed periodontal probing (periodontal probe: UNC-15, Hu-Friedy Mfg. Co., Inc., Chicago, USA), on six selected teeth of three individuals, and these probing depths were compared to those done by the gold standard on the same evening. For ethical and logistical reasons, it was not possible for evaluators to be calibrated on patients with severe chronic periodontitis. However, one of the three individuals that were measured by the evaluators had probing depths that were greater than or
equal to 5 mm. The gold standard (GS) for dental probing in this study was a Periodontist (H.C.T.), with over 25 years of experience in the field.

In the second session, the evaluators attended a fifteen minute presentation given by the same trainer, which reviewed the definitions and techniques involved in data collection. Each evaluator then demonstrated the oral swab technique intra-orally on another evaluator, while being observed by the trainer. The oral swabs were then taken to the lab for oral PMN quantification. Next, each evaluator performed periodontal probing on the same six teeth of the same three individuals from session one. These probing depths were compared to the probing depths that had been performed by the same gold standard on the evening of the second session (inter-rater reliability), and those performed by the evaluator at the first session (intra-rater reliability).

A summary sheet of the study protocol was given to the residents on the second training session to allow for review of definitions prior to their clinical rotations. The same summary sheet was also available in the operating room for reference. Where possible, the trainer also attended the first clinical rotations for all evaluators to ensure similarity of assessment for the periodontal indices.

**Patient Selection**

Patients attending the Mount Sinai Hospital Dental Program for Persons with Disabilities in Toronto, Ontario, Canada, for comprehensive dental care served as potential participants for this study. At the time of initial consultation and subsequent recall examination, each patient
has a comprehensive medical and dental history review, as well as an oral examination, which includes an assessment of dental hard and soft tissues, including periodontal health status. Depending on the extent and severity of disease, treatment needs, and level of cooperation assessed according to the Frankl Behaviour Rating Scale (Frankl, Shiere, & Fogels, 1962; see Appendix 1), patients may require dental examination and treatment under GA. The patient is then put on a prioritized waiting list, currently twelve months long, to have comprehensive dental treatment under GA in the operating room. Based on a chart review of patients that are on this waiting list and planned to have dental treatment under GA, a review of the medical and dental history was conducted to identify potential patients using the following inclusion and exclusion criteria.

**Inclusion Criteria**

1. Patients aged greater than or equal to 18 years.
2. Patients that received Frankl Behaviour Ratings of Negative and Definitely Negative at the most recent clinical examination.
3. Informed consent obtained from patient, parent, legal guardian, and/or designated substitute decision maker.

**Exclusion Criteria**

1. Patients taking medications associated with gingival hyperplasia (i.e. anticonvulsant Dilantin, calcium channel blockers, immunosuppressants).
2. Patients who revealed gingival inflammation or disease due to causes other than plaque accumulation.
3. Patients who had been treated with systemic steroids or immunosuppressants within 30 days of the dental appointment.

4. Patients with diseases related to altered neutrophil function or levels (e.g. autoimmune or immune deficiency disease, neutropenia).

5. Patients who had an edentulous arch (maxillary or mandibular), or less than 14 clinically visible teeth on the last available odontogram.

Patients were further excluded from this study on the day of the general anaesthetic appointment if the anaesthetic procedure was difficult, in order to minimize the time that the patient was under GA and to maximize patient safety. Patients were also not included if the operating room was running behind schedule, to ensure that data collection would not further delay hospital staff, or result in cancellation of another patient that was scheduled later the same day.

**Obtaining Consent**

Eligible patients or their parents, guardians, and/or substitute decision makers were sent a letter of invitation, research information, pre-stamped envelope, and consent form by mail (see Appendix 2). If the consent form was not returned in the allotted time, a follow-up telephone call was made. If participation was declined, no further contact was made. Consent was further re-affirmed by the trained evaluator on the day of the appointment. An overview of the study patient flow is available in Appendix 3.
Operating Room Algorithm

The GA for the dental patient was provided by the hospital anaesthesiologist. After naso-tracheal intubation and standard draping, a Molt mouth prop (Molt Mouth Gag Adult Size, Hu-Friedy Mfg. Co., Inc., Chicago, USA), was used to maintain mouth opening, and the oral cavity and oropharynx were suctioned. Data collection was then performed as follows:

1. Acquisition of Oral Swab Data

A cursory oral examination was followed by the acquisition of the oral swab. The oral swab data was collected first to limit alterations in the gingival crevicular fluid and potential blood transfer to the swab. A sterile oral swab (MedPro Sterile Cotton Tipped Applicators 6”, AMG Medical Inc., Montreal, Canada), was traced in a continuous motion at a 45 degree angle towards the gingival sulcus until the gingiva was noted to blanch, on the buccal surfaces of the maxillary dental arch from first permanent molar to the contralateral first permanent molar. The swab head was also rotated periodically to ensure coverage of the entire swab head. If an edentulous space was encountered then the swab was lifted off and then approximated on the next tooth in sequence. Another sterile oral swab was traced in the same manner on the mandibular arch, resulting in two oral swabs per patient. Each swab was placed in an individual Eppendorf tube (Advantech Research Products, Mississauga, Canada), containing 0.5mL of 0.9% sterile saline to create a solution, and transported to an off-site lab for processing as described later.
2. Acquisition of Periodontal Examination Data

The periodontal examination consisted of recording conventional parameters on six selected teeth, tooth numbers 16, 21, 24, 36, 41, 44 (Ramfjord, 1959). If the selected tooth was not present, the adjacent and most similar tooth was substituted. The following clinical parameters were recorded: gingival pocket depths including 6-point probing of selected teeth (periodontal probe: UNC-15, Hu-Friedy Mfg. Co., Inc., Chicago, USA), bleeding on probing, recession, mobility, plaque index, calculus index, modified gingival index, and VAS for gingival inflammation, using a standardized method for data collection. Definitions of the clinical parameters used in this study are noted in Appendix 4. Depending on the parameter measured, the 6 or 36 individual tooth scores for each variable were averaged, resulting in a mean score for each parameter.

The gingival health status of patients was categorized based on probing depth measurements from 36 tooth points according to the following classification:

Healthy: all probing depths ≤ 3 mm

Gingivitis: all probing depths ≤ 4 mm or ≤ 2 sites that are 5 mm

Mild Periodontitis: ≥ 3 to 6 sites that are ≥ 5 mm

Moderate Periodontitis: ≥ 7 to 18 sites that are ≥ 5 mm

Severe Periodontitis: ≥ 19 sites that are ≥ 5 mm
3. Clinical and Radiographic Examination

The soft tissues of the oral cavity were examined for pathology, followed by a hard tissue examination of teeth that were present for caries. Radiographic examination then followed based on the clinical findings.

4. Dental Treatment

Review of the complete clinical and radiographic examinations was followed by treatment planning and throat pack placement, scaling and root planing with ultrasonic instrumentation, dental prophylaxis, and then restorations and/or extractions as per the individualized dental treatment plan.

5. Oral Neutrophil Quantification

Oral swabs were transported to an off-site laboratory (University of Toronto, Fitzgerald Building Room 241, 150 College Street, Toronto, Ontario, Canada). Swabs were stored at 4°C to preserve the cells prior to transportation and until processed. Each oral swab had been contained in an individual Eppendorf tube containing 0.5 mL of 0.9% sterile saline, resulting in solution. Oral swab solutions were then placed in the centrifuge (Hettich Rotina 35R, Kare Scientific, Edmonton, Canada), at 2500 rotations per minute for 10 seconds at 21°C. The swabs were then removed from the tube while squeezing the swab head, resulting in PMN lysis and a MPO-containing solution. Twenty milligrams of ABTS (AO; Sigma Chemical, Burlington, Ontario, Canada), was dissolved in 3.6 milliliters of phosphocitrate buffer to produce a 1X concentrated solution. Hydrogen peroxide (45.6 microliters) was
then added to double distilled water (3.952 milliliters) to produce a homogenous solution. For each sample, 50 microliters of ABTS solution followed by 50 microliters of hydrogen peroxide solution were combined to allow for the characteristic blue colour change. Finally, each sample was placed in one or two wells of a 96 well plate, and the absorbance measured using the FLUOstar Optima microplate reader (BMG LabTech, Germany). The absorbance was measured at 420 nanometers light for ten cycles at 180 seconds per cycle. Absorbance measures obtained from each well were averaged, and the PMN concentration was calculated from a standard curve, obtained from blood PMN quantification in a parallel investigation (M. Glogauer, personal communication, July 7, 2010). Using this standard curve, the concentration can be read for any unknown sample given its absorbance reading, using the equation for the slope of the line of best fit, resulting in the PMN counts obtained per swab for each patient. The slope of the line used in this study to calculate oral PMN counts was 

\[ y = 41025x - 6494.8 \]

where \( x \) = absorbance and \( y \) = total number of PMNs.

After the completion of dental treatment, the throat pack was removed, the patient was extubated and then monitored in the post-anaesthetic care unit, and then dismissed with an appointment for follow-up in the dental clinic.

**Follow-up Appointment**

Every patient was scheduled for a follow-up and recall examination at the Mount Sinai Hospital Dental Clinic after the GA appointment, according to the patient’s usual dental
recall schedule (i.e. at 3 or 6 month intervals). At the recall examination, consent for participation in the study was re-affirmed by the trained evaluator. A review of the medical and dental histories was done, in addition to clinical extra-oral and intra-oral examinations. The oral swab technique described earlier (see the Acquisition of Oral Swab Data section above), was then completed for each dental arch. Swabs were typically performed by using the non-dominant hand to support the patients’ head and retract the lips, and using the dominant hand to collect the swabs as per the study protocol. Most patients required protective stabilization by dental staff and attending caregivers. Periodontal examination was performed and conventional gingival parameters that were assessed at this appointment were the same as those collected while under GA, except for parameters which required dental probing, namely plaque index, calculus index, modified gingival index, mobility, VAS for gingival inflammation, and bleeding on brushing. Definitions of the clinical parameters used in this study are noted in Appendix 4. Finally, scaling and root planing with ultrasonic instrumentation or hand instruments, and/or dental prophylaxis with rotary instruments or toothbrushing was conducted depending on the level of cooperation.
Statistical Analyses

All statistical analyses in this study were calculated by IBM SPSS Statistics 20 software (Chicago, USA).

Calibration Analyses

Inter-examiner reliability testing was conducted, to ensure reliability of the periodontal probing measurements when compared to the gold standard. Intra-examiner reliability testing was also carried out to ensure reliability within the individual. Reliability testing was accomplished using the intra-class correlation coefficient (ICC), and ICC scores of 0.61 or greater (i.e. substantial agreement), were considered as an appropriate level of agreement. An ICC score of 0.60 or less would necessitate re-training of the evaluator.

Patient Analyses

Non-parametric analyses were used in this study because all variables demonstrated non-normal distributions. Spearman’s Correlation was used to assess the correlation between PMN counts and periodontal variables at GA and recall. The Wilcoxon Signed Ranks Test was used to evaluate the effect of periodontal treatment at GA on the periodontal and PMN variables obtained at recall. An Analysis of Covariance (ANCOVA) was used to evaluate potential influencing factors on PMN levels. Statistical tests were interpreted at the 5% significance level (2-tailed). Finally, descriptive statistics were used to assess the feasibility (ease of data collection) of the oral swab technique on the uncooperative special needs patients at recall.
RESULTS

Calibration Analyses and Reliability Testing

Of the 14 residents scheduled to be in the operating room during the study period, 11 residents completed the calibration exercise, consisting of 3 hospital dental residents and 8 paediatric dental graduate residents. Seven of the eleven residents were scheduled on clinical rotation when participating patients were assessed under GA, and four of the eleven residents were available for recall assessments. Table 1 illustrates the intra-class correlation coefficients (ICC) for the 7 trained evaluators, who demonstrated excellent or substantial inter-rater reliability in their measurements when comparing sessions 1 and 2. Table 2 shows the ICC for the 7 evaluators, to assess for inter-calibration reliability. In this case, the participant number represents the person that was measured, and the ICC score represents the agreement between examiners when assessing that participant. Again, evaluators demonstrated excellent or substantial inter-rater reliability in their measurements of the 6 participants. It is also interesting to note that all groups improved in their measurements at the second session, except in the case of measuring participant 3. However, even then the ICC reveals substantial inter-calibration reliability, and none of the evaluators required re-training.

Oral Neutrophil Quantification at Calibration Sessions

Oral PMNs were quantified from nine residents during the calibration sessions. These residents ranged in age from 24 to 34 years, and consisted of 3 males and 6 females. Medical histories for all residents were non-contributory, and gingival health status was measured via
probing depths classified according to the description above (see the Acquisition of Periodontal Examination Data section). Five residents were healthy, while 4 had gingivitis. A summary of resident characteristics is illustrated in Table 3.

**Patient Recruitment**

During the study period, 101 patients had dental treatment under GA at the Mount Sinai Hospital, and the patient flow and reasons for exclusion from this study are represented in Table 4. Fifty-eight patients (57%) consented to participate in this study. Nineteen patients were excluded from the study after chart review, and therefore were not sent letters of invitation to participate in the study. Most of these were due to the lack of evaluator availability in the operating room, despite efforts to maximize available evaluators during the calibration exercises. Nine patients were excluded if the anaesthetic procedure was difficult, or if the operating room had scheduling delays. Some patients were not assessed if an evaluator could not attend as scheduled, or if the case was cancelled entirely. Patient recruitment was a challenge, due to the patient population involved. Most patients were unable to provide consent for themselves, and instead consent was provided by parents, caregivers, group home managers, or substitute decision makers, who usually did not reside or attend appointments with the patient. Thus, consent had to be obtained before the GA appointment. Eight decision-makers did not receive the consent form in time prior to the scheduled GA appointment. Eighteen decision-makers opted not to participate and most did not provide a reason. It is suspected that the paragraph contained within the consent form - “In case you are harmed in this study”, caused some decision-makers to decline participation in this investigation. Though there were no foreseeable risks or harms as a result of the study
technique or protocol, this paragraph had to be included in the informed consent form at the behest of the Hospital Research Ethics Board. In any case, since caregivers were not required to provide reasons for non-participation, it can only be speculated at this time as to why some did not participate.

**Demographics**

The mean age of patients that were assessed under GA was 31.3 ± 11.3 years, consisting of 31 (63%) males and 18 (37%) females. Participant age, Frankl behaviour ratings, sex, and total number of teeth at the time of GA and recall appointments can be found in Table 5, and are consistent for the two groups. All patients assessed under GA had some extent of developmental delay as shown in Figure 1. Thirty-four patients were rated as Definitely Negative at their last clinic appointment, and 15 were rated as Negative. None were in the positive range, as seen in Figure 2. In contrast, at the recall assessments seven patients were noted to be Positive, which may be due to the differences in responses to treatment being attempted before GA appointments (e.g. procedure requiring local anaesthesia compared to toothbrushing at recall). Differences could also be due to patient-specific factors, where the patient could be having a “good day” or “bad day”, translating into differences in Frankl behaviour rating scores. Twenty-five patients (51%) were non-verbal. Many patients also had concurrent medical conditions, including seizure disorder (n = 21), autism (n = 13), and cerebral palsy (n = 13), as the most common conditions. A complete list of medical conditions noted in the participating patients’ medical histories is shown in Table 6. Table 7 illustrates the complete list of medications that were noted in the patients’ medical histories. The data showed that 80% of patients were taking some type of daily medication.
Prevalence of Gingival Inflammation in Patients with Special Needs

According to the classification of gingival health status as used in this study, only one patient in the uncooperative patients with special needs cohort was found to be healthy. About 45% of the patients had gingivitis, while an equal proportion of patients were assessed as having mild (22.5%) or moderate (22.5%) periodontitis. A small proportion of patients (8%) had severe disease. The distribution of patients’ gingival health status is represented in Figure 3.

Loss to Follow-up

Of the 49 patients assessed at the GA appointment, 19 (39%) were lost to follow up. A summary of reasons for loss to follow-up and number of patients involved is available in Table 8. Four patients did not return because they are on an annual GA schedule, due to very poor cooperation. Three patients did not attend recall appointments at the Mount Sinai Hospital Dental Clinic and opted to continue regular follow-ups at their community dentist. One patient was made edentulous during the GA appointment, and one patient attended the ambulatory clinic at an un-scheduled appointment time and thus, the recall examination was performed by an un-trained evaluator and so study-specific data was not collected for analysis. One patient passed away prior to their scheduled recall appointment, and of course could not be seen. Three patients were scheduled outside of the study period, and six patients “re-scheduled” outside of the study period, where “re-scheduled” may refer to cases where the patient did not attend a confirmed appointment (i.e. “no show”), or re-scheduled in advance of the confirmed appointment. Interestingly, of the 30 patients that did attend recall, the same number of patients “re-scheduled” within the study period to allow for assessment. In other words, a total of 18 (38%) patients did not attend the originally scheduled recall
appointment. Figure 4 represents the time interval at which patients presented for recall, and illustrates that all but one patient attended the recall appointment within or after 4 months. Patients who did not return for follow-up were only included in the GA appointment data set.

**Recall Patient Exclusions for Neutrophil Counts**

As previously noted, thirty (61%) patients returned for recall examination. Of these 30 patients, three patients were excluded from data analyses involving PMN counts, for reasons described below. Hence, when examining the recall patient neutrophil data, the sample size used was 27 rather than 30.

For one patient, oral swab data were not collected by the trained evaluators in light of the clinical circumstances as follows. This patient had severe developmental delay, was non-verbal, and had concurrent medical conditions noted in the health history. The patient constantly wore a helmet with full face shield due to a history of self-biting as well as of biting others. The helmet was maintained in place with three separate locks. During the recall appointment, the patient was restrained by three people, including care workers and dental staff while the helmet was un-locked and removed and dental assessment and toothbrushing were performed by the trained evaluator. Though the trained evaluator commented that oral swabs could have been performed despite the poor cooperation, it was decided not to proceed in order to maximize the amount of time available for the dentist while the helmet was off. Interestingly, the toothbrush that was used on both the maxillary and mandibular arches was swabbed by two swabs, and measurable PMN counts were
obtained. However, these PMN counts were not included in the study as the appropriate swab technique or study protocol had not been followed.

For two patients, oral swabs were performed at recall but PMN counts were not obtained due to failure of the computer associated with the FLUOStar OPTIMA microplate reader. Both patients had been assessed on the same day, and the one-time computer failure resulted in loss of data for both patients. Because the ABTS colour reaction is time-sensitive, the patient samples could not be salvaged once the computer error was noted.

Correlation of Periodontal and Neutrophil Variables for Assessments under GA
The correlation between clinically obtained periodontal measurements and PMN variables for assessments under GA, using the Spearman Correlation Coefficient ($r_s$) are shown in Table 9. Total PMN counts obtained from the maxillary and mandibular dental arches are listed both separately and combined. Most of the relationships do not show statistically significant results because the number of teeth that were swabbed was not considered in these calculations. However, as shown in Table 10, multiple statistically significant results were actually demonstrated when the number of teeth that were measured using swabs was controlled for in the calculations. This has basic face-validity since PMNs are collected from specific sites along the gingival sulcus. Therefore, the surface area covered and the PMNs absorbed onto the swab must depend upon the number of teeth present in the measured area. Hence, this illustrates the importance of controlling for number of teeth present when interpreting the oral inflammatory load of patients based on the numbers of collected PMNs.
It is also noteworthy that consistent relationships for most periodontal variables were shown with PMN counts obtained from the swabs, whether obtained from the maxilla or mandible. Also, the number of PMNs obtained from the maxilla and mandible correlated significantly with the total maxillary and mandibular PMN values ($r_s = 0.902$ and $0.956$ respectively, $p < 0.01$), indicating that it might be possible in the future to take samples only from one arch, thus simplifying the test method even more for this difficult to manage population.

The periodontal parameters are based on the mean score of the whole mouth rather than just the maxilla or mandible separately, therefore the total PMNs per tooth of the combined swabs would reveal the true relationships between the variables and will be evaluated further. Spearman’s correlation allowed for comparisons between periodontal parameters and oral PMN counts and demonstrated positive correlations as seen in Table 10. For measures using the periodontal probe, positive correlations were noted for mean probing depths ($r_s = 0.286$, $p < 0.05$), number of probing depth sites $\geq 5$ mm ($r_s = 0.350$, $p < 0.05$), and gingival inflammation category ($r_s = 0.294$, $p < 0.05$), indicating that the PMN assay may be helpful to identify cases of inflammation where patients fall into the conventional categories of periodontitis. There was also a positive correlation between the calculus index and PMN counts ($r_s = 0.385$, $p < 0.01$), indicating the presence of a higher inflammatory load. It is interesting that a positive correlation between PMN counts and tooth mobility was found ($r_s = 0.467$, $p < 0.01$). This is important because of all periodontal parameters used for assessment of prognosis, mobility is one of the most significant ones since excessively mobile teeth are usually removed regardless of what other measures might show. Therefore, measurement of PMNs using the PMN assay may be helpful in measuring past and clinically
relevant disease activity in the population of patients with special needs. Lastly, the modified gingival index also showed a positive relationship \((r_s = 0.285, p < 0.05)\), indicating that the PMN assay positively correlated to the subjective impression of the dental examiner when evaluating inflammation based on overall gingival condition.

There were no statistically significant differences found for the following variables: number of probing depth sites \(\geq 4\) mm, plaque index, bleeding on probing, and VAS for gingival inflammation. This may be as a result of these periodontal variables having consistently higher scores, which may overestimate the presence of gingival disease when compared to levels of PMN cells, which were likely under-estimated as will be described later.

**Correlation of Periodontal and Neutrophil Variables for Assessments at Recall**

The correlation between clinical periodontal measurements and PMN counts made at recall examination is represented in Table 11. There were no statistically significant relationships apparent in this assessment. This could be due to the decreased number of PMNs, which may not allow for findings of association. However, the significant relationship indicated before between the individual swabs and the total inflammatory load while controlling for number of teeth is maintained in this data set \((r_s = 0.954\) and \(0.947\) respectively, \(p < 0.01)\), signaling that performing one swab per person could be sufficient for periodontal assessment.

**Comparing Periodontal and Neutrophil Variables Obtained at GA and Recall**

There was a statistically significant difference in mean modified gingival index, calculus index, mobility, VAS, and total PMNs controlling for number of teeth \((p < 0.05)\), when
comparing outcomes both before (i.e. at GA) and after treatment (Wilcoxon Signed Ranks Test; Table 12). The difference in PMN counts before and after treatment indicates that the PMN assay may be used to monitor responses to treatment. It also emphasizes that the treatment provided at GA allowed for a significant improvement in the extent of periodontal inflammation as measured by conventional periodontal parameters and oral PMN levels. The mean plaque index did not change significantly when comparing pre- and post-treatment values, and remained consistent over time. This indicates the presence of an undisturbed biofilm or plaque due to continued poor oral hygiene measures.

Factors Influencing Oral Neutrophil Counts

An Analysis of Covariance (ANCOVA) was done to assess the factors influencing oral PMN counts at recall, while adjusting for baseline PMN counts and age (Table 13). Notably, there was a significant difference in PMN counts when differentiating for the severity of developmental delay. In fact, patients with severe developmental delay had almost double the number of PMNs at recall when compared to those with mild or moderate developmental delay. An ANCOVA was also performed to examine the influence of the Frankl behaviour rating scale, age, sex, and extent of developmental delay, controlling for baseline levels of PMNs, on the PMNs collected at recall examination. The results (in Table 14) indicate that the extent of developmental delay was the only significant factor to influence the number of PMNs collected at follow-up (F(2) = 3.816, p < 0.05). Finally, another ANCOVA was done to examine the influence of Frankl behaviour ratings, age, sex, and extent of developmental delay, on the noted difference in PMNs counts before and after treatment. Again, the severity of developmental delay was the only confounding factor that contributed to changes
in this model (see Table 15). This is consistent with the finding that patients with severe
developmental delay have poor cooperation (see Figure 5), and so accordingly, they cannot
maintain oral hygiene independently, and tend to have poor oral hygiene.

**Feasibility of Oral Swab Data Acquisition**

Despite the lack of cooperativity in this population (see Figure 2), it was possible to use the
oral swabs on 100% of the patients that attended recall and were assessed while awake. As
noted before, one patient did not have the swabs done by the evaluator, but the evaluator did
note that it would have been possible to obtain swabs had they been attempted. Importantly,
the skill-set required to obtain the swabs for each dental arch was noted to be the same as
would be required to perform an oral exam and/or toothbrushing in this population.

In fact, breakage of the swab was the only test-related complication, and was encountered in
10/98 cases at GA and 4/58 cases at recall examination; or 14 cases in total. In 13/14 cases,
swab breakage occurred on the wooden aspect due to operator pressure, and did not cause
trauma to the patient or examiner, or interfere with data collection. The swabs used in this
study were 6 inches long, and could have been easily broken with finger pressure. This could
be remedied by using shorter swabs (i.e. 3 inches), so that the finger pressure would be
applied along a shorter length and require greater force to result in breakage. In one case at
recall, the patient bit on the swab after data collection was completed, resulting in breakage
of the swab head into the patient’s mouth. The swab head was retrieved in one piece using
the evaluator’s fingers and dental instruments. Again, no trauma was caused to the patient
(or evaluator) as a result of this incident.
Patient-related factors that led to difficulties in use of the oral swab were also noted by the evaluators and included patient movement or resistance resulting in decreased visibility of tooth surfaces. Some swabs were acquired after multiple breaks rather than one continuous motion. This may have resulted in the same tooth being swabbed twice (over-estimation of PMNs), or some tooth surfaces not being swabbed at all (under-estimation of PMNs). Lip smacking and tightness of oral musculature were also noted as challenges to oral swab acquisition at recall, resulting in the swab contacting the labial or buccal mucosa. Despite the limitations due to cooperation, it was possible to obtain measurable oral PMN counts from all of the swabs taken at the recall appointments.

**Correlation of the VAS with Traditional Periodontal Measures and PMN Counts**

As a point of interest, the VAS for gingival inflammation as measured at GA and recall was compared to the traditional periodontal variables and the PMN counts obtained by the oral swabs. Table 16 demonstrates a moderately positive correlation of the VAS with measures obtained via periodontal probing. The plaque index, calculus index, modified gingival index, and bleeding on brushing parameters also demonstrated positive correlations with the VAS at GA and recall. With the exception of the calculus index, parameters that were measured at both GA and recall consistently had statistically significant correlations with the VAS. Notably, mobility and PMN counts did not show statistically significant correlations with the VAS at GA or recall examination.
DISCUSSION

High Prevalence of Gingival Inflammation in Patients with Special Needs

As expected, the prevalence of gingival inflammation in the uncooperative special needs patients in this cohort was found to be quite high, where a conventional measurement of disease, as well as assessment of PMN levels at one point in time (at GA) demonstrated a high prevalence of disease and high inflammatory load. These results point to the importance of diagnosis of a very prevalent condition in this particular population of patients, where until now, there were no reliable diagnostic tools for identification of periodontitis; at least not until such patients were placed under GA, by which time there might be inadequate time or preparation for treatment, or disease may have progressed to a hopeless state. As described earlier, the failure to identify and treat periodontitis adequately and the persistence of a high inflammatory load can have negative oral and general health consequences.

As far as we know, this is the first study to have measured the prevalence of disease in adult patients with special needs, where a periodontal examination with numerous indices was conducted on patients who were defined as uncooperative, and where the population of interest did not consist of a majority of patients with Down syndrome (see Anders & Davis, 2010, for a recent systematic review of oral health in persons with disabilities). Therefore, the paucity of dental literature in this regard does not allow for comparisons to be made, but indicates that further studies in this area would be invaluable to recognize, manage, and hopefully prevent periodontal diseases in this at-risk population group.
Positive Treatment Outcomes in Uncooperative Patients with Special Needs

A profound and unexpected finding was made when comparing the measured clinical parameters of gingival inflammation, including traditional periodontal parameters and PMN levels, before treatment (i.e. at GA) and after treatment (i.e. at recall). **A significant improvement in all of the measured clinical parameters (except plaque index), and a lower inflammatory load was revealed – a treatment effect that was discernible despite a longer than ideal recall interval (greater than 4 months in most cases).** The positive treatment outcomes that were noted as a result of one session of non-invasive periodontal treatment (scaling and root planing with ultrasonic instruments), are especially encouraging for the clinicians involved in treating this patient population everyday.

In this study, the mean plaque index did not change significantly when comparing pre- and post-treatment values, and remained consistent over time. This indicates the presence of an undisturbed biofilm or plaque due to poor oral hygiene measures. Though these results are consistent with findings from patients with chronic periodontal disease (Cobb, 1996; Drisko, 2001; Cobb, 2002; Suvan, 2005), similar studies have not been done in uncooperative patients with special needs. In the face of poor oral hygiene, the provision of regular dental care is often met with a sense of inevitable deterioration of the oral condition. At best, it is hoped that dental treatment may prolong the certain and eventual loss of teeth in this patient population. However, this study has shown that **the provision of dental treatment as provided at the Mount Sinai Hospital Dental Program for Persons with Disabilities is effective, and moreover provides a direct benefit in reducing the oral inflammatory load.** The difference in PMN counts before and after treatment also indicates that PMNs
respond to treatment and that the PMN assay may be used to monitor treatment needs and responses to therapy, which could not be measured before in the uncooperative special needs population.

**Correlation of Periodontal and Neutrophil Variables for Assessments under GA**

For measures using the periodontal probe, positive correlations were noted for mean probing depths, number of probing depth sites ≥ 5 mm, and gingival inflammation category, indicating that the PMN assay may be helpful to identify cases of inflammation where patients fall into the conventional periodontitis categories. The calculus index correlated positively with PMN counts, indicating the presence of a higher inflammatory load consistent with the presence of calculus where more gram negative bacteria would be expected (Socransky & Haffajee, 1992). It is interesting that mobility had the strongest positive correlation, which is an index that occurs when disease is present and clinical attachment loss has already occurred. Therefore, the PMN assay may be helpful in measuring past disease activity. Lastly, the modified gingival index was also positive, indicating that the PMN assay correlated to the subjective impression of the dental examiner when evaluating overall gingival condition.

As described earlier, the number of probing depth sites ≥ 4 mm, plaque index, bleeding on probing, and VAS for gingival inflammation did not have statistically significant relationships. One explanation for this is that these types of periodontal variables generally produce higher scores, which may overestimate the presence of gingival disease when compared to PMN levels, which were likely under-estimated as will be discussed later.
Nevertheless, with PMN counts it is possible to obtain an understanding of disease activity versus signs of disease. This could also speak to the value of the PMN counts as a measure of gingival inflammation, where the conventional measures of periodontal assessment are lacking as noted previously. This area of the analyses also demonstrated the importance of controlling for the number of teeth present when interpreting the oral inflammatory load of patients based on the number of collected PMNs, and also indicated that measuring PMN levels from one arch may be sufficient to identify the patient’s gingival health status.

**Correlation of Periodontal and Neutrophil Variables for Assessments at Recall**

An assessment of the correlation between PMN counts and periodontal parameters at recall did not reveal any statistically significant relationships. The lack of correlations could be due to the fact that by the time of recall, which occurred after periodontal therapy (scaling and root planing), there was a marked reduction in PMN counts. Moreover, given a response to treatment, multiple clinical periodontal measures would have been recorded as ‘0’. This is consistent with studies showing a reduction in leukocytes after treatment, attributable to a reduction of the bacterial load in the periodontal pocket. Leukocytes may still be present in the pocket after treatment due to residual bacteria in the environment, or due to cellular activities related to the healing process (Boretti, Zappa, Graf, & Case, 1995). Our study is also consistent with another study showing a decrease in clinical indices and MPO levels after periodontal treatment, but without significant associations at follow-up with the clinical parameters (Smith, Hinrichs, & Melnyk, 1986). In this case, it was suggested that the narrow range of values for the clinical indices may not allow for an association to be detected between MPO activity and clinical variables. The lack of association may also occur because MPO levels reflect factors other than those associated with the currently used clinical
parameters of periodontal health status, which are descriptors of disease history as opposed to
disease activity (Smith, Hinrichs, & Melnyk, 1986), and which have also failed to
demonstrate significant relationships with systemic markers of inflammation (Beck &
Offenbacher, 2002).

Severity of Developmental Delay Influences Gingival Inflammation

Another notable finding in this study was the contribution of developmental delay severity to
the results, where the extent of developmental delay had a significant influence on oral PMN
counts and extent of gingival inflammation. This is consistent with findings in the literature,
where patients with severe developmental delay have poor cooperation, and have poor oral
hygiene, due to an inability to maintain oral hygiene independently and/or lack of
cooperativity to allow for oral health care needs to be met (Gabre, Martinsson, & Gahnberg,
1999; Lindemann, Zaschel-Grob, Opp, Lewis, & Lewis, 2001). About 5% of patients with
special needs will require dental treatment under GA to manage their behaviour
appropriately, and to allow for provisions of optimal dental care (Roeters & Burgersdijk,
1985; Milam, 1986). The importance of assessment in this small but considerably under-
served proportion of the special needs population is undeniable. This further highlights the
importance of diagnosis and timely and appropriate management of disease in a population
that is at significant risk for oral disease and its systemic consequences (Teng et al., 2002;
Sigal & Sigal, 2006), and underscores the importance of this study and further studies in this
field.
Acquisition of Oral Swab Data is Feasible

The oral swab technique for procurement of oral PMNs and assessment of inflammatory load is non-invasive and safe. When compared to the periodontal probe, the oral swab does not enter the gingival sulci, avoids the risks associated with being bitten due to application on the buccal surfaces only, and provides minimal trauma to the dental hard and soft tissues if bitten, the latter being common when examining or treating patients with special needs. This study has effectively demonstrated that it was possible to acquire the oral swabs on uncooperative patients with special needs while awake in the ambulatory dental clinic. As would be expected, patient-related factors led to some noted difficulties in oral swab performance (e.g. patient movement or resistance), yet this is consistent with the challenges faced while performing an oral examination or toothbrushing in this group of patients. Despite the limitations due to cooperation, it was exciting to find that it was indeed possible to obtain measurable oral PMN counts from all of the swabs performed at the recall appointment.

Correlation of the VAS with Traditional Periodontal Measures and PMN Counts

Though not a focus of this study, but as a point of interest, the VAS for gingival inflammation as measured at GA and recall was compared to the traditional periodontal variables and the PMN counts obtained by the oral swabs. As noted earlier, the use of the VAS in this manner is not noted in the literature, but is often the only periodontal-type measure that can be obtained in the limited time available for examination of the uncooperative patient in the dental clinic. The VAS for gingival inflammation provides a subjective yet quantitative measure of inflammation, as in the case of the conventional
periodontal indices, and in this study the VAS was noted to positively correlate with the multiple indices that were recorded. Therefore, it could be argued that measurement of the VAS could replace the multiple other indices when performing recall examinations, thereby providing the same level of information regarding the patients’ gingival condition. However, it should also be noted that the VAS did not correlate well to PMN counts, which importantly provides an objective and quantitative measure of inflammation. Again, this could speak to the value of the PMN assay which provides reliable and reproducible information about disease activity, and demonstrates that the PMN assay is reflecting information (i.e. disease activity and extent of gingival inflammation or total inflammatory load), that is different from the information obtained via conventional periodontal parameters.

POTENTIAL LIMITATIONS

Patients with Down Syndrome

Periodontal disease is significantly prevalent in patients with Down syndrome for multiple reasons, including neutrophil dysfunction (Morgan, 2007). As noted in the exclusion criteria for this study, patients with altered neutrophil levels were not eligible for participation in this study. However, patients with Down syndrome were included (4 patients in total), to allow for an appropriate sampling of the special needs population at the Mount Sinai Hospital Dental Clinic. Interestingly, though it would be expected that the inclusion of patients with Down syndrome could confound or obscure the results for prevalence of disease, it was observed that the one and only patient that was found to be healthy in this study was a patient with Down syndrome. Two patients had gingivitis, and one patient had severe periodontal disease. Thus, the inclusion of patients with Down syndrome contributes important
information about disease distribution and these results were therefore combined with the other patient data.

**Patients Taking Medications**

Various medications have been noted in the literature to significantly affect gingival disease, specifically anticonvulsant Dilantin, calcium channel blockers, and immunosuppressants (Armitage, 1999; Mariotti, 1999; Kinane & Marshall, 2001). As noted in the exclusion criteria for this study, patients taking these medications were not eligible for participation in this study. Albeit rare, it has been noted that other medications may impact gingival disease (Anderson, Rapley, & Williams, 1997), and MPO levels (Frimat et al., 1997). However, patients taking medications other than those specifically noted in the exclusion criteria were included to allow for a true representation of the patient population at Toronto’s Mount Sinai Hospital Dental Program for Persons with Disabilities, and specifically the uncooperative patients with special needs cohort.

**Other Sources of Peroxidases**

The oral cavity is a source of many peroxidases, with contributions from salivary glands and cellular components (Castagnola et al., 2011). The colourimetric reaction used in this study is a reaction that involves peroxidase and is not specific to myeloperoxidases from PMN granules. Therefore, it may be argued that this could have led to an over-estimation of PMN counts. However, the PMN is the most abundant source of MPOs (Klebanoff, 2005), and thus has been cited as an indicator for PMN activity, quantity, and tissue inflammation (Cao & Smith, 1989). Also, any contribution of the oral biofilm to the PMN counts may be
considered to be consistent for the pre- and post-treatment swabs, as the high plaque index noted at GA did not show a significant change at follow-up. In addition, healthy control samples of saliva using the oral rinse technique have shown that PMN levels are barely detectable (M. Glogauer, personal communication, July 7, 2010), indicating that the levels of salivary peroxidase are negligible and do not impact the PMN assay. In spite of this, efforts were made to limit the contribution of salivary peroxidases, via suctioning of the oral cavity and retraction of lips during oral swab acquisition.

Loss to Follow-Up

Of the 49 patients assessed at the GA appointment, a significant proportion (39%) was lost to follow-up. As previously noted, half of the patients that were lost to follow-up did not attend their regularly scheduled appointment, and 38% of patients in this study overall also did not attend their initially scheduled appointment. Missed and/or infrequent appointments are common in this population for a number of reasons. Patients may not be able to attend due to distances involved in travelling to the clinic, which can often be greater than one hour for one way of travel due to difficulties in accessing dental care in the local community. Limitations may also include vehicle availability, reliance on public transportation, weather or traffic delays, illness, and poor cooperation (i.e. not sitting in the car or not leaving the vehicle on arrival to the clinic). In many cases, caregivers are simply overwhelmed by appointments and so it is understandable that patients may not be able to attend regularly scheduled recall appointments. The loss to follow-up and consequent reduction in sample size for the recall data set may account for the lack of statistically significant results when comparing conventional clinical parameters of gingival inflammation with PMN counts obtained at
recall. It is important to note that these patients have subsequently been seen for on-going care, but not at the ideal recall interval or within the study period.

**Plaque Barrier**

The magnitudes of correlations in this study are lower than expected and were likely limited due to the poor oral hygiene and high plaque index measures noted in all of the patients before and after treatment. A significant plaque barrier was noted during GA and recall appointments and may have prevented absorption of cells onto the oral swab – leading to underestimation of the inflammatory load. Though the biofilm would have also contained pathogens and PMNs within it, the plaque aggregate that was collected on the swab was discluded from the samples that were measured using the FLUOstar Optima microplate reader. Pre-measurement wipe of excess plaque and debris was considered prior to the study to allow for better access to the gingival sulcus during data collection, but could cause bleeding in the presence of gingival inflammation – leading to exaggerated results of cell counts and therefore was not done. Despite this limitation, it was possible to obtain a measurable level of PMNs from the oral swabs at the GA and recall appointments, and it was possible to ascertain a difference between the swabs when PMN counts were compared pre- and post-treatment.

**Diagnostic Assessment**

Ideally, this study would have validated the PMN assay using diagnostic assessments, including calculations of sensitivity, specificity, positive and negative predictive values, and evaluation of a receiver operator characteristic (ROC) curve. However, these calculations
require a similar proportion of patients in the healthy and diseased categories to allow for appropriate assessment. As discussed earlier, in this study only one patient was found to be healthy. Therefore, the results in this study do not lend themselves to diagnostic calculations, and so were not performed. Future studies with a greater sample size and variant population gingival health status would allow for diagnostic tests for the PMN assay.

**Lack of Control Group**

This study design did not include a control group for comparison of PMN counts, and could limit the interpretation of results. Though a healthy population was measured in this study via oral PMN quantification of the residents participating in the calibration exercises, this is not appropriate for comparison with the study population due to differing sample sizes and population types, and so was not done. Though it is important to note that a review of the PMN counts obtained from the resident group illustrates an appreciable difference in PMN counts when compared to the special needs population, it would be interesting to compare healthy and cooperative special needs groups with the uncooperative special needs population for appropriate comparison in future studies.

**Multi-Examiner Approach**

The periodontal and oral swab assessments were performed by 11 calibrated residents, including graduate paediatric dental and hospital dental residents, over the course of the study. This could be interpreted as a limitation due to the potential source of variation which could obscure positive findings. On the other hand, the fact that significant findings were found in this study despite the multi-examiner approach emphasizes the robustness of results.
obtained by the PMN assay. Alternatively, this can be viewed as a positive feature of the study protocol, since the PMN assay using oral swabs is meant to be used for multiple practitioners with minimal training and calibration.

**One Piece of the Puzzle**

Recognizing the complex nature of periodontal disease pathogenesis illustrates the importance of analyzing the results of a test that looks at one component of disease (PMN presence or activity), within the backdrop of the entire clinical picture to allow for individualizing test results to the patient. Though the information obtained from the PMN assay regarding inflammatory load are encouraging, they should be evaluated in the context of the overall gingival health status as assessed by the currently standard periodontal examination. A greater number of PMNs could indicate more severe inflammation, or could be due to the interaction of PMNs with subgingival plaque microbes (Smith, Hinrichs, & Melnyk, 1986). Increased MPO levels can indicate PMN activity and the degree of gingival inflammation, but do not specifically indicate periodontitis (Cao & Smith, 1989). It is difficult to interpret the significance of a single study that evaluates levels of any particular marker of inflammation. Nevertheless, promising results from smaller studies need to be confirmed in large, randomized controlled trials. The potential advantage of salivary analyses in aiding the diagnoses of systemic disease indicates that further studies are justified in this field (Kaufman & Lamster, 2002). The goal of being able to differentiate sites that are vulnerable to progress to periodontal disease is still elusive. In this study, identification of gingival inflammation has been shown to be possible using MPO levels to quantify PMNs. However, discriminating between sites that will progress to periodontal disease may not be
possible until destruction has occurred. Thus, risk assessment, decisions about treatment intervention, and monitoring the results of the treatment may be the best solution for now (Fine & Mandel, 1986). Therefore, the PMN assay will serve as a valuable tool in providing quantitative information that will aid in the diagnosis of gingival health status in uncooperative patients with special needs.

**CLINICAL SIGNIFICANCE**

Besides the advantage of providing an objective and quantitative measure of the gingival status in a safe, non-invasive, and atraumatic fashion, in the special needs population the PMN assay could reveal total inflammatory load, guide treatment decisions and recall intervals, provide screening to prioritize treatment times for GA based on extent of disease, and allow for monitoring of responses after treatment is delivered. Perhaps most importantly, because the PMN assay correlated to the subjective impression of the dental examiner, the PMN assay may be more appropriately used by medical professionals or nurse practitioners to recognize disease and allow for appropriate referral to the dental team. Appropriate diagnosis would guide the clinician to specific treatment needs and direct individualized treatment plans, leading to a state of improved or optimal oral health in patients with special needs. The ultimate goal is that by providing treatment that leads to improved oral health, we will ultimately reduce patients’ risks for other general health problems including diabetes, cardiovascular diseases, and pulmonary infections.

In the general population, the PMN assay could allow for site-specific measurement of inflammation, monitoring of treatment responses, and reveal total inflammatory load. It may
also serve as a simple monitoring tool for other at-risk populations for oral disease such as the medically compromised or patients with long hospital admissions. Of course, further studies are required to firmly establish the value of the PMN assay in the aforementioned functions.

**FUTURE DIRECTIONS**

In the future, research is required to calibrate the color reaction to allow for a standard colour guide on how the colours reflect extent of disease (e.g. how dark does the sample solution need to be in order to be considered as moderate versus severe periodontitis) in this population. This would enable the PMN assay to move out of the lab setting and allow for a quick and standardized test at the chair-side level. As previously noted, studies similar to the present investigation in other at-risk groups for oral disease (e.g. medically compromised, long hospital admissions), could be helpful to allow for screening and recognition of disease in a non-invasive manner. It would also be interesting to investigate the potential use of the toothbrush to carry PMNs into the sample solution, which is often the only instrument used for diagnosis and treatment while the uncooperative patient is awake, thereby eliminating the extra step in performing the oral swab. This could be useful in cases where the total inflammatory load is important rather than site-specific measures. In line with the current literature in the medical field, it would be interesting to evaluate the potential relationship between oral PMN counts derived from the oral swab and systemic measures of inflammation (e.g. CRP). It is the inflammatory and host responses, and not the clinical signs of periodontitis that are currently used to assess gingival health status, which are
associated with systemic conditions (Teng et al., 2002), and could be useful in identification of patients prone to the ‘inflammatory syndrome’.

As recognized in this study, diagnosis has direct treatment implications. It would be valuable to investigate measures that may alleviate the high prevalence of disease as diagnosed in the uncooperative special needs population, such as more intensive treatment (i.e. anti-microbial or surgical treatment interventions), and/or frequent treatment (i.e. frequent recalls and maintenance therapy), in order to address the high neutrophil levels collected by the PMN assay. In addition, further research is required to evaluate how long the noted positive treatment effect persists, the ideal treatment or recall interval to allow for maintenance of the improved oral condition in this population, as well as to delineate the effects of intensive treatment on long-term periodontal health in this population.
CONCLUSIONS

1. All of the patients with special needs who were assessed with the oral PMN assay and conventional methods of periodontal measurement demonstrated gingival inflammation, which was influenced by the severity of developmental delay.

2. The measure of gingival inflammation obtained by the PMN assay (oral neutrophil quantification) positively correlated with measures of gingival inflammation as determined by conventional periodontal parameters.

3. The oral swab technique has the potential to provide information regarding the presence of disease, allow for monitoring of disease progression and treatment response, and aid in anticipating future disease.

4. Performing the swab on one arch may be sufficient to indicate total inflammatory load.

5. A single treatment intervention demonstrated a reduction in most periodontal variables, despite prolonged recall intervals.

6. It is feasible to use the PMN assay to assess gingival inflammation in the uncooperative special needs population.

Finally, this study has shown that oral PMN counts derived from swabs correlate significantly with conventional parameters of gingival inflammation and provide a standardized method for clinical assessment, thus overcoming the problem of subjectivity encountered with conventional measures, and finally allows for routine and reliable measurements for diagnosis and management of periodontal diseases in the special needs population.
FIGURES
Figure 1: Severity of Developmental Delay in Patients Assessed under GA (n = 49) and at Recall Examination (n = 30)

<table>
<thead>
<tr>
<th>Severity</th>
<th>Under GA</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>At Recall</td>
<td>2</td>
<td>7</td>
<td>21</td>
</tr>
</tbody>
</table>
Figure 2: Frankl Behaviour Rating Score in Patients Assessed Prior to GA (n = 49) and at Recall Examination (n = 30)

<table>
<thead>
<tr>
<th></th>
<th>Definitely Negative</th>
<th>Negative</th>
<th>Positive</th>
<th>Definitely Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under GA</td>
<td>34</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>At Recall</td>
<td>7</td>
<td>16</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 3: Prevalence of Gingival Inflammatory Diseases in Patients Assessed under GA (n = 49)
Figure 4: Time Elapsed (in months) Between GA and Recall Appointments (n = 30)
**Figure 5: Relationship Between Severity of Developmental Delay and Frankl Behaviour Rating Score Prior to GA Assessment (n = 49)**

<table>
<thead>
<tr>
<th>Severity of Developmental Delay</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>6</td>
</tr>
<tr>
<td>Severe</td>
<td>7</td>
</tr>
<tr>
<td>Definitely Negative</td>
<td>0</td>
</tr>
<tr>
<td>Definitely Negative</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
</tr>
</tbody>
</table>
### Table 1: Intra-Class Correlation Coefficients (ICC) for Intra-Calibration Analyses of Examiners 1 to 7 and Gold Standard (GS)

<table>
<thead>
<tr>
<th>Examiner</th>
<th>GS</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC</td>
<td>0.693</td>
<td>0.689</td>
<td>0.662</td>
<td>0.642</td>
<td>0.762</td>
<td>0.724</td>
<td>0.810</td>
<td>0.637</td>
</tr>
</tbody>
</table>
Table 2: Intra-Class Correlation Coefficients (ICC) for Inter-Calibration Analyses of Participants 1 to 6 and Gold Standard (GS), at Sessions 1 and 2

<table>
<thead>
<tr>
<th>Participant</th>
<th>GS</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session 1 ICC</td>
<td>0.823</td>
<td>0.608</td>
<td>0.625</td>
<td>0.804</td>
<td>0.774</td>
<td>0.657</td>
<td>0.706</td>
</tr>
<tr>
<td>Session 2 ICC</td>
<td>0.840</td>
<td>0.750</td>
<td>0.676</td>
<td>0.697</td>
<td>0.820</td>
<td>0.676</td>
<td>0.738</td>
</tr>
</tbody>
</table>
Table 3: Descriptive Characteristics for Residents Assessed at Calibration Sessions with the PMN Assay (n = 9)

<table>
<thead>
<tr>
<th>Resident Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (years)</td>
<td>27.8 ± 3.0</td>
</tr>
<tr>
<td>Sex (n)</td>
<td>Male = 3</td>
</tr>
<tr>
<td></td>
<td>Female = 6</td>
</tr>
<tr>
<td>Gingival Health Status (n)</td>
<td>Healthy = 5</td>
</tr>
<tr>
<td></td>
<td>Gingivitis = 4</td>
</tr>
<tr>
<td>Mean Total Number of Teeth Swabbed</td>
<td>22.6 ± 1.9</td>
</tr>
<tr>
<td>Mean Total Number of Neutrophils</td>
<td>7925.33 ± 5314.71</td>
</tr>
</tbody>
</table>
Table 4: Patient Flow During Recruitment and Reasons for Exclusion from this Study

<table>
<thead>
<tr>
<th>Patient Flow and Reasons for Exclusion</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients that had GA</td>
<td>101</td>
</tr>
<tr>
<td>Number of patients that consented</td>
<td>58</td>
</tr>
<tr>
<td><strong>Patients not eligible after chart review (n = 19)</strong></td>
<td></td>
</tr>
<tr>
<td>Epidermolysis bullosa</td>
<td>1</td>
</tr>
<tr>
<td>Patient having GA for specific treatment only</td>
<td>4</td>
</tr>
<tr>
<td>Edentulous maxilla</td>
<td>1</td>
</tr>
<tr>
<td>Age &lt;18 years old</td>
<td>1</td>
</tr>
<tr>
<td>No evaluator present</td>
<td>12</td>
</tr>
<tr>
<td><strong>Consenting patients excluded on GA date (n = 9)</strong></td>
<td></td>
</tr>
<tr>
<td>Difficult anaesthesia</td>
<td>2</td>
</tr>
<tr>
<td>Schedule delays</td>
<td>2</td>
</tr>
<tr>
<td>No trained evaluator available</td>
<td>3</td>
</tr>
<tr>
<td>Patient case cancelled</td>
<td>2</td>
</tr>
<tr>
<td><strong>Consent form not sent to decision maker (DM; n = 8)</strong></td>
<td></td>
</tr>
<tr>
<td>Recent addition to GA schedule</td>
<td>4</td>
</tr>
<tr>
<td>Not delivered to DM by intermediate</td>
<td>4</td>
</tr>
<tr>
<td><strong>Decision maker declined participation (n = 18)</strong></td>
<td></td>
</tr>
<tr>
<td>“Don’t want longer GA time”</td>
<td>1</td>
</tr>
<tr>
<td>“Don’t want additional stress during recall time”</td>
<td>4</td>
</tr>
<tr>
<td>“Want to be present during data collection”</td>
<td>1</td>
</tr>
<tr>
<td>“Don’t want to review long consent form”</td>
<td>2</td>
</tr>
<tr>
<td>No reason provided</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 5: Descriptive Characteristics for Patients Assessed under GA (n = 49) and at Recall (n = 30)

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Patients under GA (n = 49)</th>
<th>Patients at Recall (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (years)</td>
<td>31.3 ± 11.3</td>
<td>32.9 ± 11.4</td>
</tr>
<tr>
<td>Sex n (%)</td>
<td>Male = 31 (63.3) Female = 18 (36.7)</td>
<td>Male = 20 (66.7) Female = 10 (32.3)</td>
</tr>
<tr>
<td>Frankl Behaviour Rating n (%)</td>
<td>Definitely Positive = 0 Positive = 0 Negative = 15 (30.6) Definitely Negative = 34 (69.4)</td>
<td>Definitely Positive = 0 Positive = 7 (14.3) Negative = 16 (32.7) Definitely Negative = 7 (14.3)</td>
</tr>
<tr>
<td>Mean Total Number of Teeth</td>
<td>25.8 ± 4.8</td>
<td>24.0 ± 4.9</td>
</tr>
</tbody>
</table>
Table 6: Concurrent Medical Diagnoses of Patients Assessed in this Study in Order of Frequency (n = 49)

<table>
<thead>
<tr>
<th>Medical Diagnosis</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-verbal</td>
<td>25</td>
</tr>
<tr>
<td>Seizure Disorder</td>
<td>21</td>
</tr>
<tr>
<td>Cerebral Palsy</td>
<td>13</td>
</tr>
<tr>
<td>Autism</td>
<td>13</td>
</tr>
<tr>
<td>Quadriplegia</td>
<td>8</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>6</td>
</tr>
<tr>
<td>Gastro-Esophageal Reflux Disease</td>
<td>5</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>5</td>
</tr>
<tr>
<td>Rare Syndromes</td>
<td>5</td>
</tr>
<tr>
<td>Down Syndrome</td>
<td>4</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>4</td>
</tr>
<tr>
<td>Asthma</td>
<td>3</td>
</tr>
<tr>
<td>Cardiac Defect</td>
<td>3</td>
</tr>
<tr>
<td>Aspiration Risk</td>
<td>3</td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>2</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2</td>
</tr>
<tr>
<td>History of Malignancy</td>
<td>2</td>
</tr>
<tr>
<td>Hearing Impairment</td>
<td>2</td>
</tr>
<tr>
<td>Visual Impairment</td>
<td>2</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>2</td>
</tr>
<tr>
<td>Iron-Deficiency Anemia</td>
<td>2</td>
</tr>
<tr>
<td>Sickle Cell Anemia</td>
<td>2</td>
</tr>
<tr>
<td>Psychosis</td>
<td>2</td>
</tr>
<tr>
<td>Diabetes Type II</td>
<td>1</td>
</tr>
<tr>
<td>Renal Impairment</td>
<td>1</td>
</tr>
<tr>
<td>Anxiety</td>
<td>1</td>
</tr>
<tr>
<td>G-tube Fed</td>
<td>1</td>
</tr>
<tr>
<td>Paraplegia</td>
<td>1</td>
</tr>
<tr>
<td>Obese</td>
<td>1</td>
</tr>
<tr>
<td>Tuberous Sclerosis</td>
<td>1</td>
</tr>
<tr>
<td>Neurologic Dystrophy</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 7: Daily Medications Taken by Patients Assessed in this Study According to Drug Category and in Order of Frequency (n = 49)

<table>
<thead>
<tr>
<th>Medications</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>17</td>
</tr>
<tr>
<td>Anti-psychotic</td>
<td>13</td>
</tr>
<tr>
<td>Vitamin (assorted)</td>
<td>13</td>
</tr>
<tr>
<td>Stool softener/laxative</td>
<td>8</td>
</tr>
<tr>
<td>Proton Pump Inhibitor</td>
<td>6</td>
</tr>
<tr>
<td>Selective serotonin re-uptake inhibitor (SSRI)</td>
<td>5</td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>4</td>
</tr>
<tr>
<td>Thyroid Hormone</td>
<td>4</td>
</tr>
<tr>
<td>Iron Supplement</td>
<td>4</td>
</tr>
<tr>
<td>β2 Adrenergic receptor agonist</td>
<td>3</td>
</tr>
<tr>
<td>Inhalational steroid</td>
<td>3</td>
</tr>
<tr>
<td>Dopamine antagonist</td>
<td>3</td>
</tr>
<tr>
<td>Oral bisphosphonate</td>
<td>3</td>
</tr>
<tr>
<td>Anti-cholinergic</td>
<td>2</td>
</tr>
<tr>
<td>Diuretic</td>
<td>2</td>
</tr>
<tr>
<td>Alpha blocker</td>
<td>1</td>
</tr>
<tr>
<td>Eye drops</td>
<td>1</td>
</tr>
<tr>
<td>Oral hypoglycemic</td>
<td>1</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>1</td>
</tr>
<tr>
<td>Hydrocortisone cream (topical)</td>
<td>1</td>
</tr>
<tr>
<td>Histamine 2 receptor antagonist</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 8: Reasons for Loss to Follow-Up

<table>
<thead>
<tr>
<th>Reasons for Loss to Follow-Up</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual General Anaesthetic Appointments</td>
<td>4</td>
</tr>
<tr>
<td>Follow-up at Community Dentist</td>
<td>3</td>
</tr>
<tr>
<td>Edentulated at General Anaesthetic Appointment</td>
<td>1</td>
</tr>
<tr>
<td>Patient Attended Un-Scheduled Appointment</td>
<td>1</td>
</tr>
<tr>
<td>Recall Scheduled Outside of Study Period</td>
<td>3</td>
</tr>
<tr>
<td>Recall “Re-scheduled”* Outside of Study Period</td>
<td>6</td>
</tr>
<tr>
<td>→ Number of Patients that “Re-scheduled” once = 5</td>
<td></td>
</tr>
<tr>
<td>→ Number of Patients that “Re-scheduled” twice = 1</td>
<td></td>
</tr>
<tr>
<td>Deceased</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total Loss to Follow-Up</strong></td>
<td><strong>19</strong></td>
</tr>
</tbody>
</table>

* “Re-scheduled” = did not attend confirmed appointment (i.e. “no show”), or re-scheduled in advance of confirmed appointment.
Table 9: Spearman Correlation Coefficients ($r_s$) and $p$ Values Comparing Periodontal Measures and Total Neutrophils for Assessments under GA (n = 49)

<table>
<thead>
<tr>
<th>Periodontal Measures</th>
<th>Total Neutrophils</th>
<th>Maxillary Swab</th>
<th>Mandibular Swab</th>
<th>Maxillary &amp; Mandibular Swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$p$</td>
<td>$r_s$</td>
<td>$p$</td>
</tr>
<tr>
<td>Number of Sites with Probing Depth $\geq$ 4 mm</td>
<td>0.182</td>
<td>0.210</td>
<td>0.169</td>
<td>0.245</td>
</tr>
<tr>
<td>Number of Sites with Probing Depth $\geq$ 5 mm</td>
<td>0.255</td>
<td>0.078</td>
<td>0.248</td>
<td>0.086</td>
</tr>
<tr>
<td>Mean Probing Depth</td>
<td>0.222</td>
<td>0.126</td>
<td>0.174</td>
<td>0.232</td>
</tr>
<tr>
<td>Mean Modified Gingival Index</td>
<td>0.332*</td>
<td>0.020</td>
<td>0.142</td>
<td>0.330</td>
</tr>
<tr>
<td>Mean Plaque Index</td>
<td>0.056</td>
<td>0.701</td>
<td>0.140</td>
<td>0.337</td>
</tr>
<tr>
<td>Mean Calculus Index</td>
<td>0.230</td>
<td>0.112</td>
<td>0.187</td>
<td>0.199</td>
</tr>
<tr>
<td>Mean Mobility</td>
<td>0.255</td>
<td>0.077</td>
<td>0.227</td>
<td>0.117</td>
</tr>
<tr>
<td>Bleeding on Probing</td>
<td>0.128</td>
<td>0.381</td>
<td>0.142</td>
<td>0.332</td>
</tr>
<tr>
<td>Visual Analog Scale</td>
<td>0.303*</td>
<td>0.034</td>
<td>0.144</td>
<td>0.324</td>
</tr>
<tr>
<td>Periodontal Disease Category</td>
<td>0.260</td>
<td>0.071</td>
<td>0.207</td>
<td>0.155</td>
</tr>
<tr>
<td>Maxillary Swab</td>
<td>1.000</td>
<td>--</td>
<td>0.677**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mandibular Swab</td>
<td>0.677**</td>
<td>&lt;0.001</td>
<td>1.000</td>
<td>--</td>
</tr>
<tr>
<td>Maxillary &amp; Mandibular Swabs</td>
<td>0.688**</td>
<td>&lt;0.001</td>
<td>0.666**</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Significant at the 0.05 level (2-tailed)  
** Significant at the 0.01 level (2-tailed)
Table 10: Spearman Correlation Coefficients ($r_s$) and $p$ Values Comparing Periodontal Measures and Total Neutrophils Controlling for Number of Teeth for Assessments under GA (n = 49)

<table>
<thead>
<tr>
<th>Periodontal Measures</th>
<th>Maxillary Swab</th>
<th>Mandibular Swab</th>
<th>Maxillary &amp; Mandibular Swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$p$</td>
<td>$r_s$</td>
</tr>
<tr>
<td>Number of Sites with Probing Depth $\geq$ 4 mm</td>
<td>0.225</td>
<td>0.120</td>
<td>0.169</td>
</tr>
<tr>
<td>Number of Sites with Probing Depth $\geq$ 5 mm</td>
<td>0.335*</td>
<td>0.018</td>
<td>0.308*</td>
</tr>
<tr>
<td>Mean Probing Depth</td>
<td>0.295*</td>
<td>0.039</td>
<td>0.239</td>
</tr>
<tr>
<td>Mean Modified Gingival Index</td>
<td>0.294*</td>
<td>0.040</td>
<td>0.259</td>
</tr>
<tr>
<td>Mean Plaque Index</td>
<td>0.182</td>
<td>0.211</td>
<td>0.174</td>
</tr>
<tr>
<td>Mean Calculus Index</td>
<td>0.356*</td>
<td>0.012</td>
<td>0.364*</td>
</tr>
<tr>
<td>Mean Mobility</td>
<td>0.391**</td>
<td>0.005</td>
<td>0.447**</td>
</tr>
<tr>
<td>Bleeding on Probing</td>
<td>0.097</td>
<td>0.509</td>
<td>0.098</td>
</tr>
<tr>
<td>Visual Analog Scale</td>
<td>0.203</td>
<td>0.161</td>
<td>0.173</td>
</tr>
<tr>
<td>Periodontal Disease Category</td>
<td>0.285*</td>
<td>0.047</td>
<td>0.261</td>
</tr>
<tr>
<td>Maxillary Swab</td>
<td>1.000</td>
<td>--</td>
<td>0.764**</td>
</tr>
<tr>
<td>Mandibular Swab</td>
<td>0.764**</td>
<td>&lt;0.001</td>
<td>1.000</td>
</tr>
<tr>
<td>Maxillary &amp; Mandibular Swabs</td>
<td>0.902**</td>
<td>&lt;0.001</td>
<td>0.956**</td>
</tr>
</tbody>
</table>

* Significant at the 0.05 level (2-tailed)
** Significant at the 0.01 level (2-tailed)
Table 11: Spearman Correlation Coefficients ($r_s$) and $p$ Values Comparing Periodontal Measures and Total Neutrophils Controlling for Number of Teeth for Assessments at Recall ($n = 27$)

<table>
<thead>
<tr>
<th>Periodontal Measures</th>
<th>Total Neutrophils Per Tooth</th>
<th>Maxillary Swab</th>
<th>Mandibular Swab</th>
<th>Maxillary &amp; Mandibular Swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$p$</td>
<td>$r_s$</td>
<td>$p$</td>
</tr>
<tr>
<td>Mean Modified Gingival Index</td>
<td>0.088</td>
<td>0.661</td>
<td>0.147</td>
<td>0.464</td>
</tr>
<tr>
<td>Mean Plaque Index</td>
<td>-0.053</td>
<td>0.793</td>
<td>-0.026</td>
<td>0.896</td>
</tr>
<tr>
<td>Mean Calculus Index</td>
<td>-0.106</td>
<td>0.600</td>
<td>-0.099</td>
<td>0.623</td>
</tr>
<tr>
<td>Mean Mobility</td>
<td>0.043</td>
<td>0.833</td>
<td>0.204</td>
<td>0.308</td>
</tr>
<tr>
<td>Bleeding on Brushing</td>
<td>0.310</td>
<td>0.116</td>
<td>0.325</td>
<td>0.098</td>
</tr>
<tr>
<td>Visual Analog Scale</td>
<td>-0.078</td>
<td>0.698</td>
<td>-0.094</td>
<td>0.641</td>
</tr>
<tr>
<td>Maxillary Swab</td>
<td>1.000</td>
<td>--</td>
<td>0.823**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mandibular Swab</td>
<td>0.823**</td>
<td>&lt;0.001</td>
<td>1.000</td>
<td>--</td>
</tr>
<tr>
<td>Maxillary &amp; Mandibular Swabs</td>
<td>0.954**</td>
<td>&lt;0.001</td>
<td>0.947**</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Significant at the 0.05 level (2-tailed)
** Significant at the 0.01 level (2-tailed)
Table 12: Mean ± Standard Deviations* and Wilcoxon Signed Ranks Test to Compare Mean Periodontal Measures Assessed under GA (n = 49, n = 30) and at Recall (n = 30)

<table>
<thead>
<tr>
<th>Periodontal Measures</th>
<th>Patients under GA (n = 49)</th>
<th>Patients under GA (n = 30)</th>
<th>Patients at Recall (n = 30)</th>
<th>Wilcoxon Signed Ranks Test** (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Modified Gingival Index</td>
<td>2.28 ± 0.71</td>
<td>2.31 ± 0.66</td>
<td>1.88 ± 0.83</td>
<td>0.015</td>
</tr>
<tr>
<td>Mean Plaque Index</td>
<td>1.72 ± 0.66</td>
<td>1.69 ± 0.63</td>
<td>1.59 ± 0.83</td>
<td>0.584</td>
</tr>
<tr>
<td>Mean Calculus Index</td>
<td>1.14 ± 0.79</td>
<td>1.05 ± 0.67</td>
<td>0.37 ± 0.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean Mobility</td>
<td>0.22 ± 0.33</td>
<td>0.26 ± 0.33</td>
<td>0.15 ± 0.27</td>
<td>0.005</td>
</tr>
<tr>
<td>Visual Analog Scale</td>
<td>6.12 ± 2.41</td>
<td>6.03 ± 2.22</td>
<td>4.20 ± 2.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Neutrophils Per Tooth (Maxillary + Mandibular Swabs)***</td>
<td>18657.44 ± 7734.87</td>
<td>18635.43 ± 5523.97</td>
<td>12171.80 ± 7849.20</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Mean and standard deviations are parametric measurements and have been reported in this table rather than median and inter-quartile ranges (non-parametric), for simplicity of visual interpretation.
** Wilcoxon Signed Ranks Test equalizes the ‘n’ in both groups. Therefore, for this test n = 30.
*** n = 27 for neutrophil counts at recall as described in text.
Table 13: Mean and Standard Error of Neutrophil Counts Obtained at Recall, Distinguished by Severity of Developmental Delay, and Controlling for Age and Baseline Levels of Neutrophil Counts under GA

<table>
<thead>
<tr>
<th>Developmental Delay Severity</th>
<th>Mean</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>5316.23</td>
<td>5253.93</td>
</tr>
<tr>
<td>Moderate</td>
<td>8157.56</td>
<td>2764.58</td>
</tr>
<tr>
<td>Severe</td>
<td>15877.77</td>
<td>2011.32</td>
</tr>
</tbody>
</table>
Table 14: Analysis of Covariance (ANCOVA) Model for Neutrophil Counts Obtained at Recall, Controlling for Baseline Levels of Neutrophil Counts under GA

<table>
<thead>
<tr>
<th>Variable</th>
<th>ANCOVA Output</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
</tr>
<tr>
<td>Frankl Behaviour Score</td>
<td>1</td>
</tr>
<tr>
<td>Developmental Delay Severity</td>
<td>2</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
</tr>
<tr>
<td>Total Neutrophils at GA</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 15: Analysis of Covariance (ANCOVA) Model for the Difference in Neutrophil Counts Obtained at GA and Recall

<table>
<thead>
<tr>
<th>Variable</th>
<th>ANCOVA Output</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>Sig</td>
</tr>
<tr>
<td>Frankl Behaviour Score</td>
<td>1</td>
<td>2.395</td>
<td>0.137</td>
</tr>
<tr>
<td>Developmental Delay Severity</td>
<td>2</td>
<td>4.514</td>
<td>0.024</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>1.302</td>
<td>0.267</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>0.305</td>
<td>0.587</td>
</tr>
</tbody>
</table>
Table 16: Spearman Correlation Coefficients ($r_s$) and $p$ Values Comparing Periodontal Measures and Total Neutrophils Controlling for Number of Teeth with VAS for Gingival Inflammation, for Assessments at GA (n = 49) and Recall (n = 30)

<table>
<thead>
<tr>
<th>Periodontal Measures</th>
<th>VAS at GA (n = 49)</th>
<th>VAS at Recall (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$p$</td>
</tr>
<tr>
<td>Number of Sites with Probing Depth ≥ 4 mm</td>
<td>0.404**</td>
<td>0.004</td>
</tr>
<tr>
<td>Number of Sites with Probing Depth ≥ 5 mm</td>
<td>0.426**</td>
<td>0.002</td>
</tr>
<tr>
<td>Mean Probing Depth</td>
<td>0.488**</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Bleeding on Probing</td>
<td>0.690**</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean Mobility</td>
<td>0.160</td>
<td>0.273</td>
</tr>
<tr>
<td>Mean Plaque Index</td>
<td>0.551**</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean Calculus Index</td>
<td>0.590**</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean Modified Gingival Index</td>
<td>0.605**</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Bleeding on Brushing</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total Neutrophils Per Tooth*</td>
<td>0.185</td>
<td>0.203</td>
</tr>
<tr>
<td>(Maxillary + Mandibular Swabs)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* n = 27 for neutrophil counts at recall as described in text.
** Significant at the 0.01 level (2-tailed)
APPENDICES
APPENDIX 1: Frankl Behaviour Rating Scale for Assessment of Cooperation in the Dental Clinic

<table>
<thead>
<tr>
<th>Definitions in this Study</th>
<th>Frankl Behaviour Rating*</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncooperative</td>
<td>Definitely Negative (--)</td>
<td>Refusal of treatment. Exhibition of extreme negativity, e.g. crying, aggression, violence.</td>
</tr>
<tr>
<td></td>
<td>Negative (-)</td>
<td>Reluctance to accept treatment. Evidence (which may be subtle) of negative attitude.</td>
</tr>
<tr>
<td>Cooperative</td>
<td>Positive (+)</td>
<td>Acceptance of treatment, compliant, and/or cooperative. May be cautious or show reservation.</td>
</tr>
<tr>
<td></td>
<td>Definitely Positive (++)</td>
<td>Good relationship with the dentist. Enjoying the appointment and engaged in dental procedures.</td>
</tr>
</tbody>
</table>

Dear Patient/Parent/Guardian:

We would like to invite you to participate in a research study that is being performed at the Mount Sinai Hospital’s Department of Dentistry called ‘Evaluation of an Oral Swab Assay as a Quantitative Measure of Periodontal Inflammation in Patients with Special Needs’. We are interested in investigating gum disease and oral health in patients with special needs using an oral swab (Q-tip™).

Please take some time to read the enclosed information. Participation in this study is voluntary. The planned dental treatment will not be affected in any way if you decide to participate in or withdraw from the study.

If you have any questions or concerns, or require additional explanation, please contact Dr. Anita Moosani at the phone number or email address provided below. Dr. Moosani will be calling you two weeks after receipt of this letter to confirm participation in this study. Please make sure that all of your questions have been answered before signing this consent form. Before you make your decision, feel free to talk about this study with anyone you wish.

If you would like to participate, please sign and return the enclosed consent form (Page 4) in the enclosed pre-stamped envelope.

Thank you very much for your time and consideration.

Sincerely,

Anita Moosani
Dr. Anita Moosani, BSc, DDS, MSc Paediatric Dentistry Candidate
Faculty of Dentistry, University of Toronto
Phone: 416-586-5145
Email: anita.moosani@utoronto.ca

Dr. Michael J. Sigal, DDS, MSc, Dip Ped, FRCDC
Professor and Head Paediatric Dentistry, University of Toronto
Director of Paediatric Dentistry Graduate Program, University of Toronto

Dr. Howard C. Tenenbaum, DDS, Dip Perio, PhD, FRCDC
Professor of Periodontology, Faculty of Dentistry, University of Toronto
Head, Division of Research, Department of Dentistry, Mount Sinai Hospital

Dr. Michael Goldberg, DDS, MSc, Dip Perio
Assistant Professor of Periodontology, Faculty of Dentistry, U of T
Head, Division of Periodontology, Dept. of Dentistry, Mount Sinai Hospital
Staff, Wasserman Oropharyngeal Pain Clinica, Mount Sinai Hospital

Dr. Michael Glogauer DDS, Dip Perio, PhD
Canadian Institutes of Health Research Group in Matrix Dynamics
Associate Professor, Department of Periodontology, Faculty of Dentistry,
University of Toronto: Cross appointed to Faculty of Medicine, U of T
Staff, Division of Research, Dept. of Dentistry, Mount Sinai Hospital

Dr. Elenia P. Lawrence, DDS, MSc, PhD
Associate Professor, Department of Biological and Diagnostic Sciences, Dental Public Health, Faculty of Dentistry, U of T

For any questions about your rights as a research participant, you may contact:
Dr. R. Heslegrave, Chair of Mount Sinai Hospital Research Ethics Board, 416-586-4875
The Research Ethics Board is a group of people who oversee the ethical conduct of research studies. They are not part of the study team.

Consent Version 3: February 8, 2011
Research Consent Form

EVALUATION OF AN ORAL SWAB ASSAY AS A QUANTITATIVE MEASURE OF PERIODONTAL INFLAMMATION IN PATIENTS WITH SPECIAL NEEDS

Principal Investigators:
Dr. Anita Moosani, Dr. Michael Sigal, Dr. Howard Tenenbaum, Dr. Michael Glogauer, Dr. Michael Goldberg, Dr. Herenia Lawrence
Mount Sinai Hospital and Faculty of Dentistry, University of Toronto

WHAT IS THE RESEARCH ABOUT?
Patients with special needs often have a high level of gum disease, which can be difficult to diagnose with the dental instruments which are typically used to detect disease in the dental office. The lack of appropriate diagnosis of gum disease prevents the delivery of optimal treatment. This is especially relevant when recognizing oral health as an important component of general health and well being. The purpose of this study is to determine if an oral swab technique can be used to measure gum disease and inflammation surrounding the teeth. Our goal is to compare the measurements obtained by the typical dental instruments and an oral swab (Q-tip™), to gather information inside the mouth in a quick and painless manner, to assess the health status of the gums in patients with special needs, and to determine if the oral swab technique could be useful in special needs patients.

WHAT IS INVOLVED?
The measurements of the gums will be performed at the same time as the scheduled appointments for dental treatment under general anesthesia and 3 month check-up at the Mount Sinai Hospital Dental Clinic, and will take an additional 5 to 10 minutes during each appointment. No additional visits to the hospital are required. First, a Q-tip™ will be used to wipe along the front surfaces of all the teeth. While the patient is under general anesthesia, measurements of the gums will also be collected with a conventional dental instrument that allows for direct measurement of the level of the gums around the teeth. The Q-tips™ will then be transported to a laboratory for processing. The patient’s dental chart will also be reviewed to collect additional information (age, medical and dental history). Participation in this study is voluntary. You may decide not to be in this study, or to be in the study now and then change your mind later. The planned dental treatment will not be affected in any way if you decide to participate in or withdraw from the study.

WHAT ARE THE POSSIBLE HARMs OR BENEFITS?
The oral swab technique is non-invasive and safe. We do not foresee the potential for adverse events or complications related to the measurements that are to be performed under general anesthesia or at the recall examination. This is a research study, so the patient will not personally benefit by participating in this study. Eventually, the results of this study may benefit future patients with special needs and/or gum disease, by determining the extent of gum inflammation and subsequent treatment needs.

IN CASE YOU ARE HARMED IN THE STUDY
If the patient becomes ill, injured or harmed as a result of taking part in this study, they will receive care. The reasonable costs of such care will be covered for any injury, illness or harm that is directly a result of being in this study. In no way does signing this consent form waive your legal rights nor does it relieve the investigators, or involved institutions from their legal and professional responsibilities. You do not give up any of your legal rights by signing this consent form.

Consent Version 3: February 8, 2011
CONFLICT OF INTEREST
The Mount Sinai Hospital Department of Dentistry will pay for the materials used in this study. The study team has an interest in completing this study. Their interests should not influence your decision to participate in this study. You should not feel pressured to join this study.

IS THIS STUDY CONFIDENTIAL?
If you agree to join this study, the study team will look at your personal health information and collect only the information they need for the study. Personal health information is any information that could be used to identify the patient and includes your name, date of birth, medical and dental history. All of this information will be kept securely in the patient’s dental chart as part of their permanent dental records. Only the study team or the staff involved in the dental care of the patient will be allowed to look at your records. Your participation in this study also may be recorded in your medical record at this hospital.

Representatives of the Mount Sinai Hospital Research Ethics Board may look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study followed proper laws and guidelines.

All information collected during this study, including the patient’s personal health information, will be kept confidential and will not be shared with anyone outside the study unless required by law. You will not be named in any reports, publications, or presentations that may come from this study. Any information about you that is sent out of the hospital will have an assigned number and will not show the patient’s name, or any information that directly identifies the patient. All data will be analyzed using an assigned number instead of using the patient names. Medical and dental records that contain identification will be treated as confidential in accordance with the Personal Health Information Protection Act, 2004.

If you decide to leave the study, the information about you that was collected before you left the study will still be used. No new information will be collected without your permission.

QUESTIONS?
If you have any questions, concerns or would like to speak to the study team for any reason, please call: Dr. Michael Sigal at 416-586-1594 or Dr. Anita Moosani at 416-586-5145.

If you have any questions about your rights as a research participant or have concerns about this study, call Ronald Heslegrave, Ph. D., Chair of the Mount Sinai Hospital Research Ethics Board (REB) or the Research Ethics office number at 416-586-4875. The REB is a group of people who oversee the ethical conduct of research studies. These people are not part of the study team. Everything that you discuss will be kept confidential.
CONSENT

I, ____________________________, am providing consent on behalf of ____________________________.

(PATIENT NAME AND/OR MYSELF)

I have received a copy of and read the Research Consent Form (Page 2-3 of this form). I understand the nature of the study, including the potential risks and benefits. I have had adequate time to consider the information. I have spoken to Dr. Anita Moosani about any questions or concerns that I have about the study, and all of my questions have been answered. I understand that I will be given a copy of this consent form, after signing it.

I understand that information regarding the patient’s personal identity will be kept confidential. I understand that the information and data collected will be used in analyzing the results of this study. I realize that by signing this document I am not waiving any of the patient’s legal rights, nor does it relieve the investigators or involved institutions from their legal and professional responsibilities.

I agree that ____________________________ may participate in the research protocol, ‘Evaluation of an Oral Swab Assay as a Quantitative Measure of Periodontal Inflammation in Patients with Special Needs’, and I understand that I can terminate this participation at any time and for any reason, without consequence on the planned dental treatment.

My consent has been given freely.

PLEASE SIGN IN THE 4 SPACES BELOW MARKED ‘X’ AND RETURN IN THE ENCLOSED PRE-STAMPED ENVELOPE.

X ____________________________
Name of Consenter (Please Print)    X ____________________________
Signature of Consenter

X ____________________________
Relationship to Patient (Please Print)    X ____________________________
Date (dd/mm/yy)

FOR OFFICE USE ONLY.

Name of Person Confirming Consent    Signature of Person Confirming Consent

Date (dd/mm/yy)
APPENDIX 3: Overview of Study Patient Flow and Evaluators

New Patient or Recall Examination
(Mount Sinai Hospital Dental Hospital Staff or Resident)

↓

Patient put on Waiting List for Comprehensive Dental Treatment under GA
(M.J.S.)

↓

Chart Review of Patients on Waiting List to Identify Potential Study Patients and
Verification of Inclusion and Exclusion Criteria
(A.M.)

↓

Research Letter of Invitation and Consent Form Mailed to Patient/Decision Maker
(A.M.)

↓

Informed Consent Obtained
Informed Consent Not Obtained

↓

Consent Re-affirmed on Treatment Date
(Trained Evaluators)

↓

Comprehensive Dental Care Performed under GA
Oral Swabs and Periodontal Examination Completed
(Trained Evaluators)

↓

Recall Examination
Oral Swabs and Gingival Assessment Completed
(Trained Evaluators)
### APPENDIX 4: Definitions of Periodontal Measures of Gingival Inflammation Used in this Study

#### Selection of Teeth According to Ramfjord Technique (Ramfjord, 1959)
- Maxillary: right first molar, left central incisor, left first premolar.
- Mandibular: left first molar, right central incisor, right first premolar.
  - If Ramfjord tooth not present, choose the adjacent and most similar tooth (e.g. substitute missing incisor for adjacent incisor).

#### Probing Depth (Newman, Takei, & Carranza, 2002)
- Measurement of the distance from the free gingival margin to the base of the periodontal pocket.
- Measured at 6 points of tooth (buccal – mesial, midline, distal; then lingual – mesial, midline, distal).

#### Modified Gingival Index (Lobene, Weatherford, Ross, Lamm, & Menaker, 1986)
<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No inflammation noted.</td>
</tr>
<tr>
<td>1</td>
<td>Slight change in texture or colour of a portion of, but not the entire marginal or papillary gingiva.</td>
</tr>
<tr>
<td>2</td>
<td>Slight change in texture or colour of the entire marginal gingiva and papilla.</td>
</tr>
<tr>
<td>3</td>
<td>Edema, redness, and/or hypertrophy of the marginal gingiva and papilla.</td>
</tr>
<tr>
<td>4</td>
<td>Significant edema, redness, and/or hypertrophy of the marginal gingiva and papilla. Spontaneous bleeding or ulceration may also be noted.</td>
</tr>
</tbody>
</table>

#### Plaque Index (Ramfjord, 1959)
<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No visible plaque.</td>
</tr>
<tr>
<td>1</td>
<td>Plaque on some of the interproximal and gingival surfaces of the tooth.</td>
</tr>
<tr>
<td>2</td>
<td>Plaque on all interproximal and gingival surfaces, and covering less than one half of the entire tooth crown.</td>
</tr>
<tr>
<td>3</td>
<td>Plaque on all interproximal and gingival surfaces, and covering more than one half of the entire tooth crown.</td>
</tr>
</tbody>
</table>

#### Calculus Index (Ramfjord, 1959)
<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No visible calculus.</td>
</tr>
<tr>
<td>1</td>
<td>Supragingival calculus below the free gingival margin, but less than 1 mm of extension.</td>
</tr>
<tr>
<td>2</td>
<td>Subgingival calculus only, or moderate amounts of supra and subgingival calculus.</td>
</tr>
<tr>
<td>3</td>
<td>Significant supra and subgingival calculus accumulation noted.</td>
</tr>
</tbody>
</table>
APPENDIX 4: Definitions of Periodontal Measures of Gingival Inflammation Used in this Study, Continued...

### Gingival Bleeding Index (Ainamo & Bay, 1975)

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Bleeding noted from probed site within 10 seconds.</td>
</tr>
<tr>
<td>-</td>
<td>No bleeding noted.</td>
</tr>
</tbody>
</table>

- Measured at 6 points of tooth (buccal – mesial, midline, distal; then lingual – mesial, midline, distal).
- Number of sites with bleeding divided by total number of sites probed x 100.

### Mobility (Miller, 1950)

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Physiologic or normal mobility.</td>
</tr>
<tr>
<td>I</td>
<td>Tooth movement that is greater than normal.</td>
</tr>
<tr>
<td>II</td>
<td>Tooth movement of 1 mm from normal, in any direction.</td>
</tr>
<tr>
<td>III</td>
<td>Tooth movement of more than 1 mm in any direction. Depression and/or rotation in the dental alveolus may be noted.</td>
</tr>
</tbody>
</table>

### Bleeding on Brushing (Defined By This Study)

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No sulcular bleeding, no bleeding onto toothbrush head.</td>
</tr>
<tr>
<td>1</td>
<td>Slight sulcular bleeding noted (&lt; 50% of sites), and slight change in color of toothbrush bristles may be noted.</td>
</tr>
<tr>
<td>2</td>
<td>Sulcular bleeding noted (≥ 50% of sites), and blood covers all toothbrush bristles.</td>
</tr>
<tr>
<td>3</td>
<td>Tendency toward spontaneous bleeding.</td>
</tr>
</tbody>
</table>

### Visual Analog Scale for Gingival Inflammation (Defined By This Study)

The intersection of an ‘X’ mark indicated by the examiner on a 100 mm line, with endpoints of ‘Absent’ and ‘Severe’ inflammation.
REFERENCES


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