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Plaque-induced gingivitis: Case definition and diagnostic considerations

Leonardo Trombelli[1,2] | Roberto Farina[1,2] | Cléverson O. Silva[3] | Dimitris N. Tatakis[4]

[1]Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara, Ferrara, Italy

[2]Operative Unit of Dentistry, University-Hospital of Ferrara, Ferrara, Italy

[3]Department of Dentistry, State University of Maringá, Maringá, Brazil

[4]Division of Periodontology, College of Dentistry, The Ohio State University, Columbus, OH, USA

Correspondence

Prof. Leonardo Trombelli, Research Center for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara, Corso Giovecca 203, 44100 Ferrara, Italy.
Email: leonardo.trombelli@unife.it

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Abstract

Objective: Clinical gingival inflammation is a well-defined site-specific condition for which several measurement systems have been proposed and validated, and epidemiological studies consistently indicate its high prevalence globally. However, it is clear that defining and grading a gingival inflammatory condition at a site level (i.e. a "gingivitis site") is completely different from defining and grading a "gingivitis case" (GC) (i.e. a patient affected by gingivitis), and that a "gingivitis site" does not necessarily mean a "GC". The purpose of the present review is to summarize the evidence on clinical, biochemical, microbiologic, genetic markers as well as symptoms associated with plaque-induced gingivitis and to propose a set of criteria to define GC.

Importance: A universally accepted case definition for gingivitis would provide the necessary information to enable oral health professionals to assess the effectiveness of their prevention strategies and treatment regimens; help set priorities for therapeutic actions/programs by health care providers; and undertake surveillance.

Findings: Based on available methods to assess gingival inflammation, GC could be simply, objectively and accurately identified and graded using bleeding on probing score (BOP%)

Conclusions: A patient with intact periodontium would be diagnosed as a GC according to a BOP score ≥ 10%, further classified as localized (BOP score ≥ 10% and ≤30%) or generalized (BOP score > 30%). The proposed classification may also apply to patients with a reduced periodontium, where a GC would characterize a patient with attachment loss and BOP score ≥ 10%, but without BOP in any site probing ≥4 mm in depth.

KEYWORDS
gingival diseases, gingival hemorrhage, gingivitis

INTRODUCTION

In this review, the term "gingivitis" applies to plaque-induced gingivitis alone, rather than non-dental-biofilm induced forms of gingivitis, which carry the relevant prefix, such as "necrotizing", "plasma cell", "viral", "fungal" or "bacterial" gingivitis. These conditions are reviewed by Holmstrup et al.[1]

Gingivitis is generally regarded as a site-specific inflammatory condition initiated by dental biofilm accumulation[2–4] and characterized by gingival redness and edema[5] and the absence of periodontal attachment loss.[6] Gingivitis is commonly painless, rarely leads to spontaneous bleeding, and is often characterized by subtle clinical changes, resulting in most patients being unaware of the disease or unable to recognize it.[7]

When compared to periodontitis, a peculiarity of plaque-induced gingivitis is the complete reversibility of the tissue alterations once the dental biofilm is removed. Notwithstanding the reversibility of the gingivitis-elicited tissue changes, gingivitis holds particular clinical significance because it is considered the precursor of periodontitis, a disease characterized by gingival inflammation combined with connective tissue attachment and bone loss. The evidence supporting the relationship between gingivitis and periodontitis stems from longitudinal studies, where development and progression of attachment loss was associated with greater baseline levels of gingival inflammation.[8–13] In contrast, sites with no or minimal progression of attachment loss over time were characterized by the consistent absence of gingival inflammation over time.[12,14–18] Overall, these observations suggest that effective long-term control of gingivitis could prevent progressive attachment loss.[13]

The established relationship between gingival inflammation and periodontitis calls for the need to establish the clinical criteria that define a gingivitis case (GC).

From gingival inflammation to gingivitis case definition

It is clear that defining and grading a gingival inflammatory condition at the site level (i.e. a "gingivitis site")[6] is completely different from defining and grading a GC (i.e. a patient affected by gingivitis), and that one "gingivitis site" does not necessarily equate to a GC. In fact, when shifting from the description of a "gingivitis site" to the identification of a GC, the classification process is complicated by the absence of clear-cut criteria that allow for discriminating a patient with a certain extent/severity of inflamed gingival sites from a periodontally healthy patient. In this respect, while clinical gingival inflammation is a well-defined site-specific condition for which several measurement systems have been proposed and validated, the concept of a GC is intended as the means to define the disease at a patient-level. Such a definition, i.e., the selection of appropriate, distinct, and valid criteria for a GC, becomes more challenging when applied to a patient who has experienced attachment loss in the past and has been successfully treated.

Although epidemiologic studies indicate consistently that gingival inflammation is a highly prevalent condition, there is heterogeneity in the reported prevalence of gingivitis (Table 1).[19–30] Even though part of this heterogeneity can be interpreted in the light of real, genuine differences in disease occurrence among studied populations, it is evident that differences among cohorts may well be related to variations in the diagnostic criteria used to define a GC. Epidemiological studies have based the GC definition on epidemiological indices (Table 1)[19–30] such as: the Community Periodontal Index of Treatment Need (CPITN/CPI); average severity

of gingival inflammation (as assessed using gingival indices or bleeding scores); average extent of gingival inflammation (assessed as the prevalence of sites with a certain gingival index or bleeding score); combinations of severity and extent measures. The majority of epidemiologic studies investigating the prevalence of periodontal diseases, including gingivitis, are based on the use of CPITN.[31,32] However, the CPITN is not a suitable tool for defining GC.[33] It is designed to screen for the presence of periodontitis, and consequently none of the clinical parameters included in the scoring system (i.e., bleeding, supra- or sub-gingival calculus, pockets) are unique to gingivitis. When using more specific indices to assess gingival inflammation, wide variations of gingivitis prevalence are recorded in relation to varying cut-off values. In general, the more extended and severe the manifestations of the disease that are considered, the less prevalent the gingivitis. In children aged 10 to 17 years, gingivitis prevalence was very high (91%) when calculated as the proportion of individuals with GI > 0, while it was very low (0.4%) when including only those with a mean GI > 1.[23] These observations reinforce the need to identify and grade a GC on specific, straightforward, and pragmatic clinical parameters that combine severity and extent thresholds to assess gingival inflammation on a dentition-wide basis.

Purpose of the review

The purpose of the present review is to summarize the evidence on clinical, biochemical, microbiologic, genetic markers as well as symptoms associated with plaque-induced gingivitis and to propose a set of criteria to define a plaque-induced GC. Such a classification should: (1) Include the necessary information on disease severity/extent for oral health professionals to assess the effectiveness of their preventive measures and treatment regimens; (2) Help set priorities for therapeutic actions/programs, with particular emphasis on their prognostic relevance (prevention of periodontitis) and impact on quality of life; and (3) Allow the undertaking of surveillance studies to monitor the prevalence and distribution of gingivitis consistently within a cohort as well as among different populations.[34]

Collectively, the following facts underscore the paramount clinical relevance of the need for GC classification: gingival inflammation is a ubiquitous and endemic finding in children and adults worldwide; destruction of the periodontal attachment apparatus is associated with only a select number of inflamed gingival sites; gingivitis is generally neither painful nor functionally destructive; and gingival inflammation (as opposed to gingivitis) may not be a disease but a variant of health.[6] Moreover, when defining the healthy condition in a periodontium with normal support, a distinction between "pristine periodontal health", defined as a total absence of clinical inflammation, and "clinical periodontal health",

TABLE 1 Prevalence of gingivitis as derived from national, large-scale epidemiological studies or reviews

Country	Study	Population	Sample size	Clinical indices to assess gingivitis	Criteria used to identify a gingivitis case	Gingivitis prevalence
United States of America	Albandar and Kingman 1999[19]	Individuals aged 30 to 90, representing approximately 105.8 million civilian, non-institutionalized Americans	9,689	BOP	Individuals with 6 or more teeth present were classified according to the following criteria: - Extensive gingivitis: 5 or more teeth (or 50% or more of the teeth examined) with gingival bleeding; - Limited gingivitis: 2 to 4 teeth (or 25% to 50% of the teeth examined) with gingival bleeding. Individuals who did not fulfill these criteria were regarded as not having an appreciable level of gingival inflammation.	32.3% (limited: 21.8%; extensive: 10.5%)
United States of America	Li et al. 2010[20]	Subjects recruited by placing advertisements in local publications	1,000	GI	Mean full-mouth GI	GI < 0.5%: 6.1% of subjects GI > 0.5: 93.9% of subjects GI≥1: 55.7% of subjects
United Kingdom	Murray et al. 2015[21]	5 to 15-year old individuals	69,318	Not reported in the review (reported only in surveys included in the review)	Not reported in the review (reported only in surveys included in the review)	About 50% of subjects had gum inflammation
Greece	Mamai-Homata et al. 2010[22]	35 to 44-year old individuals	1,182	CPI	Highest CPI score = 1 (gingival bleeding)	16.2%
Romania	Funieru et al. 2017[23]	10 to 17-year old individuals	1,595	GI	Prevalence of gingivitis: proportion of any GI mean score > 0 Extent of gingivitis: site prevalence – proportion of gingival surfaces affected by gingivitis Prevalence of gingival bleeding: proportion of any gingival bleeding [score 2 and 3 of the GI] present in at least one gingival surface	Gingivitis prevalence: 91%

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T A B L E 1 (Continued)

Country	Study	Population	Sample size	Clinical indices to assess gingivitis	Criteria used to identify a gingivitis case	Gingivitis prevalence
Sweden	Norderyd et al. 2015[24]	Randomly selected individuals in each of the age group of 3, 5, 10, 15, 20, 30, 40, 50, 60, 70 and 80 years	1,010	GI	GI = 2 or 3	Mean % of sites with gingivitis ranged between 1.8% to 19.5% depending on age cohort
Hungary	Hermann et al. 2009[25]	Dentate or partially edentulous adults	4,153	CPI	Highest CPI score = 1 (gingival bleeding)	8%
China	Zhang et al. 2010[26]	Adults with ≥ 20 teeth	1,143	GI	Mean GI	GI≥ 1: 82.2%
India	Kundu et al. 2011[27]	Individuals aged 15 years or more	22,366	CPI	Highest CPI score = 1 (gingival bleeding)	4.3%
Australia	Australian Research Center for Population Oral Health 2009[28]	Individuals aged 15 years or more	4,967	GI	Mean GI≥ 2	19.7%
Argentina	De Muniz 1985[29]	7-8 and 12–13 year-old individuals	2,279	CPI	CPI = 1	2.7%-27.2% (depending on age cohort)
Algeria, Benin, Burkina Faso, Cap Verde, Djibouti, Egypt, Ethiopia, Ghana, Kenya, Lesotho, Libya, Malawi, Mauritius, Morocco, Namibia, Niger, Nigeria, Seychelles, Sierra Leone, Somalia, South Africa, Sudan, Tanzania, Zaire, Zimbabwe	Baelum and Scheutz 2002[30]	15 to 44-year old individuals	Reported in each study included for review	CPI	Highest CPI score = 1 (gingival bleeding)	0 to 52% (depending on the Country/study)

BOP: bleeding on probing; CPI: Community Periodontal Index; GBI: gingival bleeding index; GI: gingival index.

characterized by an absence or minimal levels of clinical inflammation, has been suggested. Overall, these considerations seem to imply that a certain amount (extent/severity) of gingival inflammation of the dentition is compatible with a patient defined as periodontally healthy.[35]

MATERIALS AND METHODS

Although specific criteria have been introduced in some epidemiologic surveys to describe gingival inflammation in large cohorts (Table 1), no definition for a GC has been universally accepted. Murakami and Mariotti[6] suggested that the extent, or the number of gingival sites exhibiting inflammation, can be described as either localized (<30% of sites are affected) or generalized (≥30% of sites are affected). They also proposed the term incipient gingivitis where, by definition, only a few sites are affected by mild inflammation, expressed as mild redness rather than edema or bleeding on probing (BOP). However, no clear definition of the most suitable parameter used to characterize the gingival inflammation on a patient-level is provided. To tackle GC identification and grading, the different parameters and methods that are currently available to define or characterize the gingival inflammation have been thoroughly reviewed.

Clinical and biological parameters used to define gingival inflammation

Clinical parameters

Clinical methods to assess the presence and severity of plaque-induced gingival inflammation at the site level are based on the evaluation of crude macroscopic changes occurring in the marginal gingival tissues during the healthy-inflamed transition.[35] The volume of the gingival crevicular fluid (GCF) has been largely adopted in clinical trials to assess the severity of gingival inflammation at site level. However, the most commonly used clinical measures for gingival inflammation mainly consist of qualitative or semi-quantitative indices based on visual assessment of gingival characteristics (edema/swelling, redness, etc.) and/or the evaluation of the tendency of the marginal gingiva to bleed upon mechanical stimulation exerted typically by a periodontal probe. These methods were first described more than 45 years ago and have not changed much since then (Table 2).[4,36–48]

In an attempt to circumvent the subjectivity of examiner scoring, non-invasive methods based on digital technologies were introduced more recently. These methods mainly aim at measuring the volumetric or color changes that occur in the gingival tissues due to plaque-induced inflammation.[49–56] Although their application would be highly desirable in the diagnosis of gingivitis, no histologic validation of these instruments is currently available. Moreover, few studies have evaluated their reliability in subjects with gingivitis.[49,54,56] While some studies reported a positive association between the gingival volume and GI changes (without reporting the statistical strength of the association),[49] other studies failed to find a significant correlation between colorimetric assessments and variations in GI.[56] Moreover, additional aspects, including need for standardized conditions for their use, restriction of colorimetric assessments to the buccal attached gingiva of anterior teeth and need for specific adjustments for colorimetric evaluations of pigmented gingival tissues in specific ethnic groups, limit the potential to apply these technologies reliably or pragmatically to define a GC.

Therefore, for the purpose of this review, the authors limited the analysis of the available clinical parameters as potential candidates to define a GC to GCF volume, gingival index (GI),[37] and gingival bleeding indices.

Volume of gingival crevicular fluid

Previous studies demonstrated that the quantification of GCF volume is a reliable and accurate indicator of gingival inflammation.[4,57,58] In 60 gingival samples retrieved from buccal sites, GCF volume increased with increasing site-specific GI. The GCF volume reflected GI values, with an evident difference between bleeding sites with moderate inflammation (GI = 2) compared to non-bleeding sites (GI < 2), and paralleled two objective measures of tissue inflammation, i.e., the percentage of inflamed connective tissue area and the inflammatory infiltrate density.[57] Experimental gingivitis studies demonstrated a clear association between GCF volume and other clinical parameters of gingival inflammation,[4] as well as the concentration of pro-inflammatory biomarkers.[58] Overall, these and other studies clearly indicate that GCF volume represents a reliable quantitative method to assess the severity of site-specific, plaque-induced gingival inflammation in the research setting. However, in clinical practice, measurement of GCF has proven to be challenging, costly and time consuming.[59] Consequently, GCF volume seems to be unsuitable to use for a GC definition that fulfills the aforementioned pragmatic criteria.

Gingival index

The GI[37] is based on the combination of visual assessment and mechanical stimulation of the marginal periodontal tissues by probing gently along the soft tissue wall of the gingival sulcus/pocket. Technically, to stimulate the gingival tissues the probe engages approximately 1 to 2 mm of the gingival margin with the probe at a 45-degree angle with moderate axial pressure. GI scores are assigned on a 4-point ordinal scale: 0 = absence of inflammation; 1 = mild inflammation – slight change in color and little change in texture; 2 = moderate inflammation – moderate glazing, redness, edema and

TABLE 2 Gingival indices. Re-adapted from: *Bessa Rebelo MA, Corrêa de Queiroz A. Gingival Indices: State of Art. In: Gingival Diseases – Their Aetiology, Prevention and Treatment, 2011 pp: 41–54. Edited by Dr. Fotinos Panagakos*

Index name (authors and year)	Instrument	Sites for assessment	Time delay (seconds)	Graded response
PMA Index (Schour and Massler 1947[36])	Visual assessment	Each gingival unit is scored. Only the labial surfaces are examined.	Not stated	P (papillary) 0 = normal; no inflammation; 1 = mild papillary engorgement; slight increase in size; 2 = obvious increase in size of gingival papilla; hemorrhage on pressure; 3 = excessive increase in size with spontaneous hemorrhage; 4 = necrotic papilla; 5 = atrophy and loss of papilla (through inflammation). M (marginal) 0 = normal; no inflammation visible; 1 = engorgement; slight increase in size; no bleeding; 2 = obvious engorgement; bleeding upon pressure; 3 = swollen collar; spontaneous hemorrhage; beginning infiltration into attached gingivae; 4 = necrotic gingivitis; 5 = recession of the free marginal gingiva below the CEJ due to inflammatory changes. A (attached) 0 = normal; pale rose; stippled; 1 = slight engorgement with loss of stippling; change in color may or may not be present.; 2 = obvious engorgement of attached gingivae with marked increase in redness. Pocket formation present; 3 = advanced periodontitis. Deep pockets evident.
Gingival Index (Löe and Silness, 1963[37])	Probe	It scores the marginal and interproximal tissues (four areas for each tooth). The bleeding is assessed by probing gently along the wall of soft tissue of the gingival sulcus.	Not stated	0 = Normal gingiva; 1 = Mild inflammation – slight change in color and slight edema but no bleeding on probing; 2 = Moderate inflammation – redness, edema and glazing, bleeding on probing; 3 = Severe inflammation – marked redness and edema, ulceration with tendency to spontaneous bleeding.
Sulcus Bleeding Index (Mühlemann and Son 1971[38])	Probe	Four gingival units are scored systematically for each tooth: the labial and lingual marginal gingival (M units) and the mesial and distal papillary gingival (P units).	Not stated	Score 0 – health looking papillary and marginal gingiva no bleeding on probing; Score 1 – healthy looking gingiva, bleeding on probing; Score 2 – bleeding on probing, change in color, no edema; Score 3 – bleeding on probing, change in color, slight edema; Score 4 – bleeding on probing, change in color, obvious edema; Score 5 – spontaneous bleeding, change in color, marked edema.

(Continues)

TABLE 2 (Continued)

Index name (authors and year)	Instrument	Sites for assessment	Time delay (seconds)	Graded response
Gingival Bleeding Index (Carter and Barnes 1974[39])	Unwaxed dental floss	The mouth is divided into six segments and flossed in the following order; upper right, upper anterior, upper left, lower left, lower anterior and lower right.	Not stated; 30 s is allowed for reinspection	Bleeding is recorded as present or absent.
Gingival Bleeding Index (Ainamo and Bay 1975[40])	Probe	Gentle probing of the orifice of the gingival crevice.	10	If bleeding occurs within 10 seconds a positive finding is recorded
Papillary Bleeding Index (Mühlemann 1977[41])	Probe	A periodontal probe is inserted into the gingival sulcus at the base of the papilla on the mesial aspect, and then moved coronally to the papilla tip. This is repeated on the distal aspect of the papilla.	Not stated	Score 0 – no bleeding; Score 1 – A single discreet bleeding point; Score 2 – Several isolated bleeding points or a single line of blood appears; Score 3 – The interdental triangle fills with blood shortly after probing; Score 4 – Profuse bleeding occurs after probing; blood flows immediately into the marginal sulcus.
Papillary Bleeding Score (Loesche 1979[42])	Wooden interdental cleaner	This is performed using a Stim-U-Dent®, which is inserted interproximally. The PBS is determined on all papillae anterior to the second molars.	Not stated	0 = healthy gingiva, no bleeding upon insertion of Stim-U-Dent® interproximally; 1 = edematous, reddened gingiva, no bleeding upon insertion of Stim-U-Dent® interproximally; 2 = bleeding, without flow, upon insertion of Stim-U-Dent ® interproximally; 3 = bleeding, with flow, along gingival margin upon insertion of Stim-U-Dent® interproximally; 4 = copious bleeding upon insertion of Stim-U-Dent ® interproximally; 5 = severe inflammation, marked redness and edema, tendency to spontaneous bleeding.
Modified Papillary Bleeding Index (Barnett et al. 1980[43])	Probe	modified the PBI index (Muhlemann, 1977) by stipulating that the periodontal probe should be gently placed in the gingival sulcus at the mesial line angle of the tooth surface to be examined and carefully swept forward into the mesial papilla. The mesial papillae of all teeth present from the second molar to the lateral incisor were assessed.	0-30	0 = no bleeding within 30 s of probing; 1 = bleeding between 3 and 30 s of probing; 2 = bleeding within 2 s of probing; 3 = bleeding immediately upon probe placement.
Bleeding Time Index (Nowicki et al. 1981[44])	Probe	Inserting a Michigan "0″ probe in the sulcus until slight resistance was felt and then the gingiva was stroked back and forth once over an area of approximately 2 mm.	0-15	0 = no bleeding within 15 seconds of second probing (i.e. 30 seconds total time); 1 = bleeding within 6 to 15 seconds of second probing; 2 = bleeding within 11 to 15 of seconds of first probing or 5 seconds after second probing; 3 = bleeding within 10 seconds after initial probing 4 = spontaneous bleeding.

(Continues)

TABLE 2 (Continued)

Index name (authors and year)	Instrument	Sites for assessment	Time delay (seconds)	Graded response
Eastman Interdental Bleeding Index (Caton and Polson 1985[45])	Wooden interdental cleaner	A wooden interdental cleaner is inserted between the teeth from the facial aspect, depressing the interdental tissues 1 to 2 mm. This is repeated four times	0-15	Bleeding within 15 s is recorded as present or absent.
Quantitative Gingival Bleeding Index (Garg and Kapoor 1985[46])	Toothbrush	Takes into consideration the magnitude of blood stains covering tooth brush bristles on brushing and squeezing gingival tissue units in a sextant	Not stated	0 – no bleeding on brushing; bristles free from blood stains; 1 – slight bleeding on brushing; bristle tips stained with blood; 2 – moderate bleeding on brushing; about half of bristle length from tip downwards stained with blood; 3 – Severe bleeding on brushing; entire bristle length of all bristles including brush head covered with blood.
Modified Gingival Index (Lobene et al. 1986[47])	No instrument (visual assessment)	Same as Gingival Index	Not applicable	0 = absence of inflammation; 1 = mild inflammation or with slight changes in color and texture but not in all portions of gingival marginal or papillary; 2 = mild inflammation, such as the preceding criteria, in all portions of gingival marginal or papillary; 3 = moderate, bright surface inflammation, erythema, edema and/or hypertrophy of gingival marginal or papillary; 4 = severe inflammation: erythema, edema and/or marginal gingival hypertrophy of the unit or spontaneous bleeding, papillary, congestion or ulceration.
Modified Gingival Index (Trombelli et al. 2004[4])	No instrument (visual assessment)	Same as gingival index, but without the bleeding on probing component.	Not applicable	0 = Normal gingiva; 1 = Mild inflammation – slight change in color and slight edema; 2 = Moderate inflammation – redness, edema and glazing; 3 = Severe inflammation – marked redness and edema, ulceration with tendency to spontaneous bleeding.
Bleeding on Interdental Brushing Index (Hofer et al. 2011[48])	Interdental brush	Inserting a light interdental brush placed buccally, just under the contact point and guided between the teeth with a jiggling motion, without force. Bleeding is scored for each interdental site.	30	Bleeding is scored as either present or absent

hypertrophy; bleeding on pressure; 3 = severe inflammation – marked redness and hypertrophy, ulceration with tendency to spontaneous bleeding. The validation of the GI comes from histological studies in humans where GI scores were significantly correlated with histological parameters of inflammation during gingivitis development;[60] specifically, the infiltrated connective tissue volume and its ratio with the volume of non-infiltrated connective tissue increased with increasing GI. Also, a higher percentage of lymphocytes and lower percentage of fibroblasts was associated with high GI scores.[60]

Since its introduction, the GI has been widely used in clinical periodontal research and, together with its modifications,[4,47] it currently represents the most widely used index of gingival inflammation in clinical trials on preventive/therapeutic strategies.

To evaluate the GI at the patient-level,[37] a GI score has to be assigned to four areas (buccal, lingual, mesial and distal) for each of six index teeth (maxillary right first molar and lateral incisor; maxillary left first premolar; mandibular left first molar and lateral incisor; mandibular right first premolar – the so-called "Ramfjord teeth"), and scores of the areas can be averaged to give the GI for the patient. The routine application of the GI in clinical practice to define a GC, however, presents potential drawbacks: 1)The GI was originally proposed to describe gingivitis in pregnant women rather than the general population, and the GI scale seems to reflect the specific gingival conditions of such individuals. For example, a score of 3 represents a tendency for spontaneous bleeding, which is a rare occurrence in the general gingivitis population in contrast to women with pregnancy gingivitis;[6] 2) Since it is based on both visual inspection and mechanical stimulation of the gingival margin, the assessment of GI will result in a time-consuming procedure when incorporated in a comprehensive, whole-mouth examination (i.e., 4–6 sites per each tooth present) to obtain data representative of the inflammatory burden of the entire dentition; and 3) Intra- and inter-examiner reliability and reproducibility of the GI, particularly the component associated with visual inspection, while reported as very good in some studies,[61] appears problematic even after calibration and training sessions in other reports.[62,63]

Gingival bleeding

Gingival bleeding was first incorporated in a clinical periodontal index in 1958.[64] Much interest was given to this clinical sign in the following years, based on evidence that during the development of gingivitis the appearance of bleeding on probing typically precedes other clinically detectable signs, such as color (redness) or volume changes (edema).[38,65] Indeed, apart from a sparse number of studies that failed to show significant differences at the histological level between bleeding and non-bleeding gingiva,[66,67] the great majority of studies found that gingival bleeding is an early and accurate sign of gingival inflammation; some studies reported that sites with gingival bleeding are histopathologically characterized by a larger and/or denser inflammatory connective tissue infiltrate than non-bleeding sites while others reported a significant reduction in inflamed connective tissue with the suspension of bleeding.[60,66,68–73] Available human histology studies have validated both BOP[40] and the bleeding component of GI (i.e., scores 2 and 3)[37] as measures of gingival inflammation. In these studies, gingival biopsies were obtained at buccal gingival sites with shallow probing depth in subjects undergoing

a 21-day experimental gingivitis trial[60] or periodontal surgery for interproximal pocket elimination.[68,74] The results showed an association between BOP and quantitative/qualitative alterations of the inflammatory infiltrate within the connective tissue, with the percentage of inflamed connective tissue being significantly greater at BOP-positive sites compared to BOP-negative sites (28.7% vs. 19.1%, respectively).[68] Similarly, the ratio between the volume densities of infiltrated and non-infiltrated connective tissue was found to be higher at sites bleeding upon probe stimulation (i.e., having a GI = 2) compared to non-bleeding sites (GI = 0 or 1). Also, a significant increase in the percentage of lymphocytes and a significant decrease in the percentage of fibroblasts were found for GI = 2 compared to GI = 0.[60]

Gingival bleeding presents additional characteristics in favor of its application in clinical practice: 1) It is an obvious, objective clinical sign that may be easily assessed and recorded;[39,68,75–79] 2) At a site level, it has been correlated with the severity of the inflammatory condition of the gingival tissues;[60,68] 3) With suitable training, it is possible for general dental practitioners to achieve and maintain high levels of inter-examiner consistency in assessing bleeding;[80] 4) It has prognostic relevance for periodontal deterioration at the site level, when persistently present during multiple observation intervals. In this respect, it has been demonstrated that BOP sites (GI = 2) have higher odds for attachment loss and exhibit greater prevalence of progressive severe attachment loss when compared to non-bleeding sites (GI = 0 or 1);[12] and 5) Patient-level (i.e., representative of the entire dentition) data on gingival bleeding can be easily derived from the site-specific measurements, e.g., frequency or proportion of bleeding sites, thus generating parameters that can be effectively used to inform and motivate the patient[41,70,71,81] as well as monitor the efficacy of preventive and treatment strategies of periodontal diseases.[82–84]

Methods to assess gingival bleeding: gingival stimulation

Varying methods have been proposed to assess gingival bleeding. Among those, the most commonly used are: BOP score,[40] scores of 2 to 3 of the gingival index[37] and the angulated bleeding index (AngBS).[4,85–87] These methods are based on a different diagnostic maneuver with respect to probing stimulation of the gingival tissues. While the probe is inserted to the bottom of the gingival sulcus/pocket with a standardized force when assessing BOP, it is used to exert a gentle pressure on the gingival margin with a specific angulation when assessing GI or AngBS. Under conditions of naturally occurring gingivitis, a significant intra-subject correlation was observed between BOP and bleeding of the marginal gingiva (i.e., GI 2 and 3).[88,89] Concordance between BOP and GI bleeding was found to be dependent on the probing depth (PD) of examined sites. While 85.4% of agreement was found for the detection

of bleeding at sites with PD > 4 mm, 77.7% of agreement was observed between absence of GI bleeding (i.e., GI ≤ 1) and absence of BOP at shallow (≤2 mm) pockets.[88] Despite their correlation, however, GI bleeding and BOP seem not to have the same potential to detect gingival inflammation and, therefore, should not be considered as equivalent parameters. In this respect, some studies reported a tendency towards higher bleeding prevalence for GI assessment compared to BOP,[88] while others reported a consistently higher (about 10%) proportion of bleeding sites when probing at the bottom of the sulcus/pocket.[89] On the basis of the finding that in young systemically healthy dental students the number of GI bleeding sites was similar to the number of BOP+ sites after a period of supervised oral hygiene, while it was double after a 21-day period of experimentally-induced plaque accumulation, it has been suggested that bleeding upon stimulation of the marginal gingiva seems to be a better indicator of early inflammatory changes in the gingival tissues when compared to BOP to the bottom of the pocket.[87] In contrast, a large scale study has confirmed that outcomes of the two stimulation approaches (marginal versus bottom of the pocket) are highly correlated (r = 0.89), with probing the bottom of the pocket resulting in 1.5-fold increase in average prevalence of bleeding-positive sites per patient.[90] Therefore, there is no consensus on the best gingival bleeding measure to incorporate in a GC definition.

Within the context of a GC definition, some practical considerations may point to probing to the bottom of the sulcus/pocket (as performed when assessing BOP) as the preferred method to stimulate and assess gingival bleeding: 1) The detection and recording of bleeding upon stimulation by a probe inserted in the gingival sulcus is a part of the comprehensive periodontal examination as included in periodontology education programs; 2) Probing to the bottom of the sulcus/pocket may diagnose the presence of gingival inflammation while simultaneously assessing other relevant clinical parameters (attachment level, probing depths), which gingival margin bleeding cannot achieve. Since a site (and thus, a patient) with gingivitis should not present with attachment loss, a single probing maneuver allows collection of the information necessary to detect the presence of both gingival inflammation and attachment loss. On the contrary, gingival bleeding assessment using GI does not incorporate the evaluation of the integrity of the periodontal support and, therefore, cannot be considered exhaustive when aiming to definitively establish a GC diagnosis, i.e., when needing to differentiate between gingivitis and periodontitis; 3) Bleeding following probing to the sulcus/pocket base is performed as part of the CPITN/CPI screening system in both clinical and epidemiological practice; and 4) The BOP score is the bleeding index that has most often been correlated with patient-related periodontal prognosis, self-reported symptoms[91] and quality of life.[35,92–94]

Methods to assess gingival bleeding: dichotomic or graded assessment

Given that the clinical assessment of gingival inflammation at a site-specific level is based on BOP, the extent of gingival inflammation in a dentition is related to the proportion of BOP+ sites. However, BOP may also be used to provide the severity of the inflammatory condition of the gingival tissues, as expressed by qualifying the bleeding tendency[42,46,95] or its timing after probe insertion.[41,44] Although useful for research purposes, it appears that the use of quantification indices to routinely qualify BOP at a site level may be time consuming, with variations in the grading scale difficult to detect during a routine comprehensive periodontal examination.[96]

Methods to assess gingival bleeding: probe/probing characteristics

The periodontal clinical signs detected through probing include bleeding tendency, PD, and clinical attachment level (CAL). Early on, it became evident that assessments of PD and CAL are subject to significant variability.[97] In fact, a large body of literature is dedicated to the technical and clinical aspects of periodontal probing as it relates to PD and CAL assessments.[98–104] The development of pressure-sensitive, controlled-force, automated, and computer controlled probes[105–113] was the result of the strong interest in determining the relationship between CAL and histologic attachment level and efforts to minimize the variability associated with probing determinations. Despite providing controlled forces, improved instrument precision, and electronic data capture, electronic probes do not offer a substantially improved measurement error.[100,114] This fact, combined with the increased time and cost associated with the use of electronic probes,[115] makes it easy to understand why manual probes remain the instrument of choice in clinical practice. There is also evidence that this lack of improved reproducibility with certain electronic probes may be related to patient discomfort, with the patient being a significant variable when determining probing reproducibility.[116]

Available data showed that probing force is a significant factor in determining BOP response. Probing force has a direct and linear effect on BOP prevalence, with forces greater than 0.25 N (25 g) increasing the risk of false-positive readings,[117–119] while use of constant force results in greater reproducibility of bleeding scores.[120] The probing force applied by different clinicians varies significantly and often exceeds the 25-g threshold.[105,121,122] From a patient perspective, greater probing forces are likely to exceed the pain threshold in healthy sites[123] and even more likely in inflamed sites.[124]

Another technique-related factor is angulation/placement of the probe, which was reviewed in the previous section.

In terms of instrument characteristics, probes with different tip diameters exhibit varying abilities to penetrate

gingival tissues.[125,126] This is consistent with the observation that thinner probes may elicit more pain during periodontal examination.[127] Although there is no consensus regarding optimal probe tip diameter specifically for BOP determination, limited evidence suggests that a probe tip diameter of 0.6 mm provides the best discrimination between diseased and healthy sites.[126]

Research has been conducted on the effect of probe tine shape (parallel, tapered, tapered ball-tipped) on PD assessment under different probing forces;[128] the results indicate that tine shape also impacts upon PD measurements. However, specific information on the impact of probe tine shape on BOP has not been reported.

In the context of probe characteristics and BOP assessment, it should be noted that commercially available probes have shown significant variation in dimensions (probe tine diameter and calibration of markings) when different samples were examined, even from the same production batch.[129–131] If millimeter markings are not relevant for BOP assessment, the probe diameter is. Although the available literature suggests that probe diameter variability has declined in more recent years, standardization of the manufacturing parameters for periodontal probes would help minimize such variability.

Although, as mentioned above, clinicians often use a probing force > 25 g,[105,121,122] with the average maximum probing force reported to be in the 50- to 70-g range,[122] such differences in force magnitude have been shown to result in consistent but moderate changes in BOP prevalence. For example, the mean BOP response when a 25- and a 50-g probing force were applied varied by 3 to 16 percentage points, depending on patient status (pre- or post-treatment, high or low BOP tendency) and study.[117–119] The lack of information in the literature on the prevalence of patients who fall within a particular mean BOP range given a specific probing force applied, combined with the fact that the aforementioned studies were based on a limited number of participants (10 to 12), makes it difficult to fully ascertain the true impact of the probing force on the categorization of patients based on their BOP response. Nevertheless, further review of the data reported from patients with optimal oral hygiene[118,119] suggests that use of a 25-g force results in a majority (∼70%) of these patients having a BOP response of ≤10%.

Methods to assess gingival bleeding: full-mouth vs. partial-mouth assessment

Although a comprehensive periodontal examination is generally based on the examination of all teeth at mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual (MB-B-DB-ML-L-DL) surfaces,[132] a partial mouth examination protocol (based on a minimum number of selected quadrants, teeth and sites representative of the entire

dentition) would be highly desirable for both patients and oral health professionals.

At present, however, the everyday clinical application of a partial-mouth examination protocol in defining the extent of gingival inflammation remains limited by the following issues: 1) Available validation data are not sufficient to identify the most accurate partial-mouth examination protocol. Although the level of agreement between partial-mouth and full-mouth examination protocols in the evaluation of the prevalence, severity and extent of gingival inflammation has been evaluated in a few studies,[133–137] there is limited information on which partial mouth protocol shows the best accuracy in representing the severity/extent of gingivitis as assessed by BOP;[137] 2) Clinical assessments to identify and grade a GC are necessarily incorporated in a comprehensive, full-mouth examination, which also aims at detecting and grading attachment loss. Although a recent systematic review has pointed out that some partial-mouth examination protocols well approximated a full-mouth protocol for prevalence, severity, and extent estimates of periodontitis,[138] their performance when applied to the periodontitis case definitions suggested by the CDC/AAP[139] or the European Federation of Periodontology[140] remains unknown. Therefore, as of now, the case definition of periodontitis (and, consequently, of a GC) remains based on the full-mouth examination of 4/6 sites per each tooth present;[141] and 3) Albeit a viable, and oftentimes, desirable approach in the research setting, the option to partially assess the dentition of a patient presenting in one's clinical practice for comprehensive examination is not really an option.

Consequently, on the basis of the available evidence and the considerations reported above, the definition of a GC should be based on the full-mouth evaluation of all sites available for examination.

Biomarkers in oral fluids

With increasing knowledge of gingivitis pathophysiology, specific biomarkers detected in oral fluids have emerged as potential candidates to help characterize and thus define a GC. Among the most promising biomarkers are inflammatory cytokines, indicators of the inflammatory host response, which can be recovered from GCF and saliva.[142,143]

GCF proteomics

Although several studies have investigated GCF proteomics under conditions of gingival inflammation, most of them concentrated on the healthy-inflamed transition at specific sites. Proteomic analyses conducted on GCF obtained from healthy sites (i.e., sites with GI = 0, PD ≤ 3 mm, attachment loss ≤1.5 mm) of periodontally healthy subjects showed that GCF proteomics is rather complex, consisting of approximately 200 distinct proteins, 57% of which were identified also in

plasma and 43% were apparently not plasma related.[144] This clearly indicates that even though serum contributes to GCF composition, GCF is an oral fluid with a distinctive proteomic profile. Moreover, this quantitative analysis of GCF showed that the dominant proteins in conditions of periodontal health were intracellular and nucleotide proteins (25%) and hydrolytic enzymes (19%).[144] Under experimental gingivitis conditions, the GCF proteomic profile of inflamed sites showed substantial changes when compared to that observed in periodontal health. In particular, only 28 proteins out of 186 identified at inflamed sites were found to be common with those detected at healthy sites.[145]

More recently, there has been a further attempt to characterize the GCF profile of a patient with gingivitis (i.e., a patient with a given amount of gingival inflammation and no attachment/bone loss) (Table 3).[146–155] Overall, these studies indicate that the GCF proteomic profile of gingivitis subjects is qualitatively and quantitatively different from that of periodontal health; more specifically, a greater number of proteins have been found in gingivitis compared to periodontal health.[153] Moreover, the amount of some proteins (e.g., IL-1b, ALP, complement factors, MMP-9, fibronectin, lactotransferrin precursors, alpha-actinin) is higher in gingivitis compared to periodontal health,[147,153] while other proteins (e.g., cystatin-B, cystatin-S) are present in lower amounts in gingivitis.[153]

Despite these reported GCF proteomic differences between periodontal health and gingivitis, the overall paucity of data on the GCF proteomic profile of gingivitis subjects, along with the heterogeneity between studies in terms of GC definition (Table 3), site selection for GCF sampling, and GCF sampling methods, as well as the practical limitations in performing such an assessment chairside in daily practice, currently eliminate the possibility to use the GCF proteomic profile as the basis for GC definition.

Salivary proteomics

Whole mouth saliva (WMS) is not only composed of major and minor salivary gland secretions but also contains mucosal transudates from all surfaces of the mouth, lymphoid tissues, oropharynx, and GCF. Saliva, a hypotonic aqueous solution that contains proteins, peptides, enzymes, hormones, sugars, lipids, growth factors and a variety of other compounds, has a complex composition.[156] Proteomic studies on human saliva revealed > 1,000 proteins and peptides.[143]

Some studies have characterized the salivary proteomic profile of gingivitis (i.e., a patient with a given amount of gingival inflammation and no attachment/bone loss) compared to periodontal health (Table 4).[146,154,155,157–160] The analyses showed that gingivitis was associated with significantly increased amounts of blood proteins (serum albumin and hemoglobin), immunoglobulin peptides and keratins,[158]

PGE2 and MIP-1α,[160] and more than double the amounts of MMP-8, MMP-9, and IL-6.[157] In periodontal health, salivary cystatins appeared to be more abundant.[158] Similarly to GCF proteomics, the use of salivary proteomics to identify a patient with gingivitis has substantial limitations, mainly due to the heterogeneity in gingivitis definition among studies (Table 4), as well as the methodology used for proteomic profiling.

Microbiologic markers

From the earliest studies of Löe and coworkers, which established the bacterial etiology of gingivitis in the 1960s,[2,3] to investigations reported in the late 1990s,[161–165] the microbiological assessment of gingivitis (and periodontitis) was based on bacterial culture, and morphological, biochemical and other targeted analyses of collected plaque samples. These studies identified several Gram-positive anaerobes (e.g., *Actinomyces viscosus, Parvimonas micra (formerly Micromonas and Peptostreptococcus micros)*), Gram-positive facultative species (*Streptococcus* spp), and Gram-negative anaerobes (e.g., *Campylobacter gracilis, Fusobacterium nucleatum, Prevotella intermedia, Veillonella parvula*) as associated with gingivitis,[166] with the flora becoming more diverse with time and the development and progression of gingivitis.[167] Efforts to identify microbiologic differences among persons with a stronger or weaker gingival inflammatory response to plaque accumulation did not find significant differences.[161] Although quantitative differences were consistently identified for targeted species among sites characterized by gingivitis and periodontitis or health,[162–164] none of the associated bacterial species were unique to gingivitis and, therefore, their presence cannot be considered pathognomonic.

The introduction in the late 90s of open-ended molecular methods and their application to the detection of microbes broadened significantly the spectrum of bacterial species associated with periodontal diseases, with many previously unidentified and/or uncultivated bacteria linked with periodontitis.[168–171] In the last few years, these molecular techniques have been applied, along with novel statistical approaches, to the study of the biofilm associated with gingivitis and compared to health and periodontitis.[172–177] These studies have demonstrated that the transition from health to disease follows the principles of primary ecological succession, with change in abundances of indigenous species, rather than acquisition of newer organisms. Even as these studies identified previously unrecognized species in gingivitis, they confirmed that the biofilms associated with gingivitis and periodontitis share most species (albeit with quantitative differences). Emerging evidence suggests that clusters of bacteria, rather than individual species, might be of use as diagnostic markers for each disease; and that bacterial functions (e.g., proteolysis, flagellar assembly, bacterial motility) may be a more robust discriminant of disease than species. While

TABLE 3 Studies comparing GCF biomarker levels in gingivitis and other periodontal conditions (i.e., health and periodontitis)

Authors	Year of publication	Population	Sites for GCF assessment	Periodontal health (H): case definition	Gingivitis (G): case definition	Periodontitis (P): case definition	Main results
Ulker et al.[146]	2008	Recruited at the Faculty of Dentistry, University of Gazi, Turkey (G = 10, H = 25)	In the G group, GCF samples were collected from four maxillary upper incisors that were affected by gingivitis.	Not reported	Not reported	–	No significant differences in the levels of cystatin C, TNF-α, and IL-1b between G and H.
Perozini et al.[147]	2010	Recruited at the University of Taubatè, Brazil (P = 12, G = 12, H = 12)	Two randomly selected teeth in each patient	According to AAP 1999 (systemically healthy with no history of periodontal disease)	According to AAP 1999 (clinical signs of inflammation without attachment loss)	According to AAP 1999 (clinical signs of inflammation with attachment loss)	In G, IL-1b concentration was significantly lower compared to P and similar to H. ALP levels in G were significantly lower than P and higher than H.
Hardan et al.[148]	2011	Recruited at Temple School University, US (P = 23, G = 18, H = 32)	4 sites (1 per quadrant) The sites were the most representative of each condition.	No CAL loss < 5 sites with GI = 2	No CAL loss ≥ 5 sites with GI = 2	≥ 4 teeth (≥ 1 tooth in each quadrant) with ≥ 1 site with CAL≥ 4 mm	GCF levels of hydrophobic aminoacids showed a significant increase from healthy to G condition. No difference in GCF levels of sulfur compounds between H and G.
Becerik et al.[149]	2012	Recruited at the School of Dentistry, Ege University, Izmir, Turkey (Aggressive P = 20, Chronic P = 20, G = 20, H = 20)	Mesio-buccal aspects of two anterior teeth	PD ≤3 mm No gingival recessions attributable to periodontitis CAL≤ 2 mm at ≥90% of sites BOP score < 10% Radiographic distance between the CEJ and bone crest ≤3 mm at > 90% of the proximal tooth sites	Varying degrees of gingival inflammation CAL ≤2 mm at ≥90% of sites Radiographic distance between the CEJ and bone crest ≤3 mm at > 90% of the proximal tooth sites	Aggressive P: CAL ≥5 mm and PD≥ 6 mm on ≥8 teeth, at least 3 of those are other than central incisors or first molars Radiographic bone loss ≥30% of the root length on affected teeth; Chronic P: CAL ≥5 mm and PD ≥6 mm in multiple sites of all four quadrants of the mouth. Moderate-to-severe alveolar bone loss present on radiographs	IL-11 total amount was significantly higher in Chronic P compared to G. No significant differences in total amounts of IL-1b, IL-6, OSM, and LIF between G and either P or H. G had elevated OSM concentration when compared to H, and significantly higher LIF concentration than Aggressive P. No significant differences in concentration of IL-1b, IL-6, and IL-11 between G and either P or H.

(Continues)

TABLE 3 (Continued)

Authors	Year of publication	Population	Sites for GCF assessment	Periodontal health (H): case definition	Gingivitis (G): case definition	Periodontitis (P): case definition	Main results
Gokul et al.[150]	2012	Recruited at the Department of Periodontics, Priyadarshini Dental College & Hospital, Chennai, India (P = 20, G = 20, H = 20)	Not reported	Clinically healthy periodontium with no evidence of disease (Ramfjord's Periodontal Disease Index = 0)	Clinical signs of inflammation with no evidence of attachment loss and radiographic bone loss (Ramfjord's Periodontal Disease Index = 1–3)	Clinical signs of inflammation with attachment loss and radiographic bone loss (Ramfjord's Periodontal Disease Index = 4–6)	TNF-α levels in G were significantly higher than H, and similar to P.
Ertugrul et al.[151]	2013	Recruited at the Faculty of Dentistry, Yuzuncu Yil University, Turkey (Aggressive P = 21, Chronic P = 21, G = 21, H = 21)	4 sites in 4 Ramfjord teeth in H and G subjects 4 BOP+ sites in 4 Ramfjord teeth in G subjects 4 BOP+ sites in 4 teeth showing the deepest pockets in the chronic and aggressive P subjects	No CAL > 2 mm No PD > 3 mm BOP score < 15% Radiographic distance between the CEJ and bone crest < 3 mm at > 95% of the proximal tooth sites	BOoP score > 50% Radiographic distance between the CEJ and bone crest < 3 mm at > 95% of the proximal tooth sites	Aggressive P: 16–30 years of age ≥ 20 natural teeth ≥ 6 incisors and/or first molars with ≥ 1 site with PD and CAL > 5 mm ≥ 6 teeth other than first molars and incisors with ≥ 1 site with PD and CAL > 5 mm Chronic P: Inflammation in the gingiva Vertical and horizontal bone loss on radiographs PD≥ 5 mm in ≥ 6 sites of at ≥ 4 single-rooted teeth with CAL≥ 4 mm	IN G, CCL28, IL-8, IL-1b and TNF-a levels Were significantly higher compared to H and significantly lower compared to Chronic P and Aggressive P.

(Continues)

TABLE 3 (Continued)

Authors	Year of publication	Population	Sites for GCF assessment	Periodontal health (H): case definition	Gingivitis (G): case definition	Periodontitis (P): case definition	Main results
Kinney et al.[152]	2014	Recruited at the Michigan Center for Oral Health Research clinic, Ann Arbor, Michigan (P = 44, G = 24, H = 15)	Mesiobuccal aspect of 8 sites. Site selection was based on group classification (in patients without periodontitis, sites with PD less than 4 mm and/or CAL less than 3 mm were ranked higher; in patients with gingivitis, sites were ranked even higher if they had BOP).	CAL < 3 mm No PD > 4 mm BOP score ≤ 20% No radiographic alveolar bone loss	CAL < 3 mm No PD > 4 mm BOP score > 20% No radiographic alveolar bone loss	≥ 4 sites with CAL > 3 mm ≥ 4 sites with PD > 4 mm ≥ 4 sites with radiographic bone loss	GCF biomarkers associated with stable and progressing cases were evaluated.
Huynh et al.[153]	2015	Patients attending the Royal Dental Hospital of Melbourne and staff at the Melbourne Dental School, Australia.	The sites chosen were the most representative of each condition.	PD≤ 3 mm BOP score≤ 5% mGI < 1 PI < 20%	BOP score > 5% mGI≥ 1 PI ≥ 20% No radiographic bone loss	≥ 2 sites with PD≥ 5 mm BOP score≥ 5% mGI≥ 1 PI I≥20% radiographic bone loss	Forty-two proteins were considered to have changed in abundance. Of note, cystatin B and cystatin S decreased in abundance from H to G and further in P. Complement proteins demonstrated an increase from H to G followed by a decrease in P.
Köseoǧlu et al.[154]	2015	Recruited at the Department of Periodontology, Faculty of Dentistry, Izmir Katip Cxelebi University, Izmir, Turkey (P = 20, G = 20, H = 20)	2 sites in 1 single- rooted and 1 multirooted tooth. In H: BOP- sites with GI≤ 1 and PD≤ 3 mm; In G: BOP+ sites with GI≥ 2 and PD≤ 3 mm; In P: BOP+ sites with GI≥ 2 and PD≥ 5 mm	No CAL loss PD≤ 3 mm BOP score < 20%	No CAL loss PD≤ 3 mm BOP score≥ 20%	≥ 4 teeth in each jaw with PD≥ 5 mm, CAL≥ 4 mm ≥ 50% alveolar bone loss in ≥ 2 quadrants BOP score > 50%	IL-35 levels in G were significantly lower than H and similar to P.

T A B L E   3   (Continued)

Authors	Year of publication	Population	Sites for GCF assessment	Periodontal health (H): case definition	Gingivitis (G): case definition	Periodontitis (P): case definition	Main results
Saglam et al.[155]	2015	Recruited at the Faculty of Dentistry, Izmir, Turkey (P = 20, G = 20, H = 20)	2 non-adjacent sites selected according to the baseline clinical measurements	PD < mm BOP score < 20% Radiographic distance between the CEJ and bone crest≤ 2 mm	PD < 4 mm BOP score≥ 20% Radiographic distance between the CEJ and bone crest≤ 2 mm	≥ 4 teeth in each jaw with PD ≥ 5 mm and CAL ≥ 4 mm BOP score > 80% ≥ 50% alveolar bone loss in ≥ 2 quadrants	The IL-37 total amount was similar between G and either H or P. IL-37 concentration was significantly lower in P compared to G and H.

ALP: alkaline phosphatase; BOP: bleeding on probing; CAL: clinical attachment level; CCL28: mucosa-associated epithelial chemokine; CEJ: cementum-enamel junction; mGI: modified gingival index; IL-1ß: interleukin 1ß; IL-6: interleukin 6; IL-8: interleukin 8; IL-11: interleukin 11; IL-35: interleukin 35; IL-37: interleukin 37; LIF: leukemia inhibitory factor; OSM: oncostatin M; PD: probing depth; PI: Plaque Index; TNF-α: tumor necrosis factor α.

these early novel findings support a gene-centric[178–182] rather than a species-centric approach to disease causation, further studies are required to better characterize such bacterial clusters and gene functions and to validate their potential use both as a diagnostic tool and as response to treatment monitoring tool.[183]

Systemic inflammation markers (CRP)

As for other chronic inflammatory diseases, the relationship between periodontal diseases (including gingivitis) and systemic levels of inflammatory markers has been evaluated. The biologic mechanisms supporting the plausibility of this association rely on the entry of pathogenic bacteria from the biofilm of periodontally diseased sites into the blood stream and on the entry into the circulation of excess local levels of host-derived inflammatory mediators.

Among the investigated biomarkers, particular attention has been paid to C-reactive protein (CRP), which is produced in response to many forms of trauma or diseases and contributes to host defense as part of the innate immune response. Studies that evaluated the association between gingivitis and serum levels of CRP universally identified gingivitis as a condition characterized by serum CRP levels which are intermediate between those measured in periodontal health and periodontitis, although differences in serum CRP levels observed between gingivitis and the other periodontal conditions did not consistently reach statistical significance in all studies.[184–186] In subjects with gingivitis, the severity and extent of gingival inflammation were evaluated for their relationship with CRP levels in serum. While in some studies CRP levels were found to be significantly positively correlated with papillary bleeding index[186] or GI,[184] other authors failed to find an association between CRP levels and GI,[185] BOP,[185,187] or the number of sextants with at least one BOP+ site.[188] Certain factors may have contributed to the hetero-geneity among these findings. First, criteria for GC definition varied greatly among studies. Second, control of potential confounders through adequate statistical analyses (e.g., multivariate models) was applied only in some studies.[187,188] Overall, the above mentioned findings seem to demonstrate that the inflammation of marginal gingival tissues determines an increase in systemic inflammation, assessed in terms of CRP levels. However, other studies have failed to demonstrate potentially relevant systemic effects during gingivitis development.[189] Therefore, the relationship between severity of gingival inflammation and severity of systemic inflammation in patients with gingivitis remains unclear.

Genetic markers

Two specific pieces of information suggest that susceptibility to gingivitis may be genetically controlled.[190,191] The first line of evidence comes from studies of patients with Down syndrome. Despite no differences in plaque accumulation rates, patients with Down syndrome, compared to age- and sex-matched genetically healthy controls, exhibit more extensive gingival inflammation and at much earlier times.[192] The second line of evidence comes from studies on twins. Michalowicz et al.[193] studied monozygous and dizygous adult twin pairs and reported that, based on ratios of within-pair variances or heritability estimates, there was a significant genetic component for gingivitis and other clinical parameters. For gingivitis, in particular, they estimated from reared-apart monozygous twins that 82% of the population variance may be attributed to genetic factors.[193] These findings provide strong support for the role of genetic make-up in gingivitis susceptibility.

Recent evidence is available evaluating whether genetic characteristics, in general, and gene polymorphisms, in particular, may contribute to exacerbated gingival inflammation in response to plaque accumulation. Since the host

TABLE 4 Studies investigating salivary biomarker levels in gingivitis and other periodontal conditions (i.e., health and periodontitis)

Authors	Year of publication	Population	Periodontal health (H): case definition	Gingivitis (G): case definition	Periodontitis (P): case definition	Main results
Ulker et al.[146]	2008	Recruited at Faculty of Dentistry, University of Gazi, Turkey (G = 10, H = 25)	Not reported	Not reported	–	No significant differences in cystatin C, TNF-α and IL-1ß levels between G and H
Ramseier et al.[157]	2009	Recruited at the Michigan Center for Oral Health Research clinic, Ann Arbor, Michigan, US (P = 49, G = 32, H = 18)	CAL < 3 mm PD > 4 mm BoP score ≤20% No radiographic bone loss	CAL < 3 mm PD > 4 mm BoP score > 20% No radiographic bone loss	≥4 sites with CAL > 3 mm ≥4 sites with PD > 4 mm ≥4 sites with radiographic bone loss	G showed levels of MMP-8 and MMP-9 that were intermediate between H and P
Da R. Goncalves et al.[158]	2011	Recruited at the School of Dentistry, Federal University of Espirito Santo, Brazil (G = 10, C = 10)	BOP score < 10% PD < 3 mm	No CAL loss BOP score > 50% PD > 3 mm in > 50% of sites	–	G was associated with increased amounts of serum albumin and hemoglobin, immunoglobulin peptides and keratins. Cystatins were more abundant in H
Kinney et al.[159]	2011	Recruited at the Michigan Center for Oral Health Research clinic, Ann Arbor, Michigan, US (P = 41, G = 23, H = 15)	CAL < 3 mm No PD > 4 mm No radiographic bone loss BOP score ≤20%	CAL < 3 mm No PD > 4 mm No radiographic bone loss BOP score > 20%	≥4 sites with CAL > 3 mm ≥4 sites with PD > 4 mm ≥4 sites with radiographic bone loss	Same cohort as Ramseier et al. 2009[157]. The paper focuses on the association of salivary biomarkers and periodontal disease progression.
Köseoğlu et al.[154]	2015	Recruited at the Department of Periodontology, Faculty of Dentistry, Izmir Katip Cxelebi University, Izmir, Turkey (P = 20, G = 20, H = 20)	No CAL loss PD ≤3 mm BOP score < 20%	No CAL loss PD ≤3 mm BOP score ≥20%	≥ 4 teeth in each jaw with PD ≥ 5 mm, CAL ≥ 4 mm ≥ 50% alveolar bone loss in ≥ 2 quadrants BOP score > 50%	IL-35 levels in G were significantly lower than H and significantly higher than P
Saglam et al.[155]	2015	Recruited at the Faculty of Dentistry, Izmir, Turkey (G = 20, H = 20)	PD < 4 mm BOP score < 20% Radiographic CEJ-bone crest ≤ 2 mm	PD < 4 mm BOP score≥20% Radiographic CEJ-bone crest ≤2 mm	≥ 4 teeth in each jaw with PD ≥ 5 mm and CAL ≥ 4 mm BOP > 80% ≥ 50% alveolar bone loss in ≥ 2 quadrants	Similar levels of IL-37 between H, G, and P

(Continues)

TABLE 4 (Continued)

Authors	Year of publication	Population	Periodontal health (H): case definition	Gingivitis (G): case definition	Periodontitis (P): case definition	Main results
Syndergaard et al.[160]	2014	Recruited at the University of Kentucky College of Dentistry, Kentucky, US (G = 40, H = 40)	No CAL ≥2 mm PD ≤4 mm BOP score < 20%	No CAL≥ 2 mm PD≤ 4 mm BOP score≥ 20%	–	Concentrations of MIP-1α and PGE$_2$ were significantly Higher (2.8 times) in G compared to H

BOP: bleeding on probing; CAL: clinical attachment level; CEJ: cementum-enamel junction; IL-1ß: interleukin 1ß; IL-6: IL-35: interleukin 35; IL-37: interleukin 37; MIP-1α: macrophage inflammatory

protein 1α; MMP-8: matrix metalloproteinase 8; MMP-9: matrix metalloproteinase 9; PD: probing depth; PGE$_2$: prostaglandin E$_2$; TNF-α: tumor necrosis factor α.

immune response is a dominant gene expression pathway during the onset and resolution of gingival inflammation, with several genes being significantly up- or downregulated,[194] particular emphasis has been placed upon evaluating the potential association between cytokine gene polymorphisms and gingival inflammation in either observational, cohort studies[195–200] or experimental gingivitis trials.[201–204] Although the available evidence suggests a role for some gene polymorphisms in determining the susceptibility to plaque-induced gingival inflammation, definitive associations between ≥1 genetic indicators and the severity of gingival inflammation are not yet available, in part because of the limited number of gene loci investigated and the small number of subjects included in pertinent studies.[205] To date, a limited number of studies have attempted to investigate the genetic profile of gingivitis and healthy cases (Table 5).[197,200,206–208] However, large-scale genome-wide association studies hold promise for the identification of genetic variations that are significantly associated with severe gingival inflammation.[209]

Emerging evidence indicates that the inflammatory response may be modulated in a dynamic way by epigenetic processes, which are heritable and reversible. In particular, the modern concepts of epigenetics imply that gene expression may be modified by environmental exposures such as diet, microbial infections, cigarette smoke, and diabetes. This implies that the genetic component of susceptibility to gingival inflammation could vary during post-natal life, without introduction of any mutations to a specific gene's DNA.[210] Diseases such as cancer, initially identified as genetic, are now known to involve both genetic and epigenetic abnormalities.[211] Even though pertinent studies are still limited in number,[212] it is reasonable to hypothesize that epigenetic modulators will be evaluated in the future for their potential impact on gingivitis.

In conclusion, when considering the pandemic distribution of gingivitis and its high prevalence in different populations, it can be hardly expected that a GC definition can be based exclusively on genetic/epigenetic profiling/susceptibility, which currently remains to be determined.

Self-reported diagnosis

Although studies on self-assessment of oral health demonstrated the validity of self-reporting on teeth present, decayed teeth, missing teeth, malocclusion and prosthetic condition, studies on self-assessment of periodontal condition revealed inconsistent results with varying levels of validity.[7] When considering gingivitis, the most investigated self-reported symptom is "bleeding from gums".[91,213–223] Several studies have validated self-reported bleeding perception with BOP scores.[91,217–219,221,222] Overall, findings seem to indicate that self-perceived bleeding (either spontaneous or evoked by different mechanical stimulations) shows high specificity and low sensitivity. In the study by Schwarz,[83] participants were asked "do you have gum problems?". Participants who self-reported "no gum problems" showed a gingival bleeding index (GBI) of 6.1%, those who self-reported "gum problem often" showed a GBI of 24.5%. Baser et al.[91] showed that 19 out of 20 dental students who presented with BOP < 10% reported no bleeding gums whereas about half of the students with gingival bleeding (i.e. BOP > 10%) correctly identified themselves as having gingival disease. In conclusion, the available data suggest that the self-assessment of bleeding does not have sufficient validity for screening individuals affected by gingivitis. Interestingly, a limited number of bleeding sites (i.e. < 10%) appears to be associated with a self-perception of periodontally-healthy conditions.

Oral health-related quality of life (OHRQoL)

Few studies evaluated the impact of gingivitis on OHRQoL.[92,93,224] In a cohort of 1,034 Thai children, Tsakos et al.[224] showed that, while the prevalence of periodontal treatment need (CPI > 0) was 97%, the perception of a condition-specific (CS) impact was limited to 27.1%

TABLE 5 Case-control studies investigating the association between gene polymorphisms and gingivitis (versus healthy controls)

Authors	Year of publication	Population	Periodontal health (H): case definition	Gingivitis (G): case definition	Investigated gene polymorphisms	Main results
Dashash et al.[206]	2006	248 whites Aged 8 to 12 years (G = 164, H = 84)	Healthy gingiva And no evidence of bleeding on probing or clinical signs of inflammation	Clinical evidence of gingivitis assessed by gingival and bleeding on probing indices	$IL-10_{-1082}$ $IL-10_{-819}$ $IL-10_{-592}$	The GCC/GCC genotype, which has been associated with increased production of IL-10, was significantly more frequent in H than in G.
Dashash et al.[197]	2007	146 whites Aged 8 to 12 years (G = 98, H = 48)	Healthy gingiva and had Neither evidence of bleeding on probing nor clinical signs of inflammation	Presence of Bleeding on probing at any site, as determined by gingival and papillary bleeding on probing indices	IL-1RN	Significant association between IL-1Ra genotype and periodontal status (H vs G). The IL-1RN*2 allele (A2) was significantly more frequent in H, and the carriage of A2 seemed to be protective against gingivitis.
Holla et al.[207]	2008	455 whites Aged 11 to 13 years (G = 272, H = 183)	GI = 0 At all 24 examined sites	Total sum of GI values at 24 examined sites≥ 4	$IL-6_{-174}$ $IL-6_{-572}$ $IL-6_{-597}$	Significant differences in haplotype frequencies between G and H. The CGA haplotype was significantly more frequent in G than in H. The IL-6 – 174C allele was more frequent in G than in H, and allele C remained a risk factor for G regardless of plaque or gender.
Vokurka et al.[200]	2009	298 whites Aged 11 to 13 years (G = 147, H = 151)	GI = 0 At all 24 examined sites	Total sum of GI values at 24 examined sites≥ 4	$MMP-9_{-1562}$ $IL-18_{-607}$	The prevalence of $MMP-9_{-1562}$ alleles was significantly higher in G compared to H. A highly significant association of the composite genotype (formed by the variants of both genes) with G was found.
Garlet et al.[208]	2012	608 whites and Afro-American/ Mulatto subjects (P = 197, G = 193, H = 218)	BOP score < 10% PD > 3 mm CAL > 1 mm	BOP > 70% ≤ 1 tooth per sextant with CAL loss ≤1 mm No history of tooth loss due to periodontitis	$IL1B_{-3954}$ $IL6_{-174}$ $TNFA_{-308}$ $IL10_{-592}$ $TLR4_{-299}$	Positive associations were found for $IL6_{-174}$, $IL10_{-592}$ and $TLR4_{-299}$

[a]BOP: bleeding on probing; CAL: clinical attachment level; gingival index; IL-1: interleukin 1; IL-1RA: interleukin 1 receptor antagonist; IL-6: interleukin 6; IL-10: interleukin 10; MMP-9: matrix metalloproteinase 9; TNF: tumor necrosis factor.

of subjects. Specificity with respect to individuals with no CS-impact among periodontally healthy subjects was 0.83. Similarly, in a sample of 1,100 12-year old and 871 15-year old Thai children, <30% of subjects had CS-impact on their quality of life related to gingivitis and calculus despite the high prevalence (about 80%) of gingivitis and/or calculus. The impact of gingivitis on children's OHRQoL was mostly at low levels of extent and intensity. However, extensive gingivitis was significantly associated with a moderate/higher level of CS-impacts.[92] In a random sample of 1,134 12-year-old Brazilian schoolchildren, gingivitis extent showed an impact on OHRQoL, with mean quality of life scores being 1.15 higher for children with ≥15% BOP+ sites than for children with < 15% BOP+ sites.[93] Extent of gingival bleeding (≥15% BOP) was significantly associated with emotional well-being, oral symptoms, functional limitations and social well-being domains.[93]

Overall, data from these studies indicate that, although highly prevalent, gingivitis has a limited impact on OHRQoL. However, gingivitis extent, in terms of BOP score, may increase the negative effects on CS and general OHRQoL. Interestingly, an increasing level of agreement between the impact of gingivitis (CPI = 1 vs. CPI = 2) on patient's quality of life and the presence of a normative need for periodontal treatment has been reported.[224]

RESULTS AND DISCUSSION

The use of BOP to define and grade a GC

Based on available methods to assess gingival inflammation, a GC could be simply, objectively and accurately defined and graded using a BOP score (BOP%).[40] A BOP score is assessed as the proportion of bleeding sites (dichotomous yes/no evaluation) when stimulated by a standardized (dimensions and shape) manual probe with a controlled (~25 g) force to the bottom of the sulcus/pocket at six sites (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, disto-lingual) on all present teeth.

BOP may be used for (i) discriminating between a healthy and gingivitis patient,[35] and (ii) classifying a GC (localized, generalized).[6] Use of BOP to identify a GC case would have the following advantages: 1) It is an objective, universally accepted, reliable and accurate clinical sign that may be easily assessed and recorded[39,68,75–79] as part of probing assessments necessary for a comprehensive periodontal examination; 2) Gingival bleeding represents a clinical sign often perceived by the patient, whereas low level of BOP% are consistent with self-reported perception of healthy gingival conditions; 3) BOP recording is user-friendly, economic, and requires minimal/no technology. With suitable training, it is possible for general dental practitioners to achieve

and maintain high levels of intra-examiner consistency in assessing bleeding;[80] and 4) Bleeding score can be effectively used to inform and motivate the patient[41,70,71,81] as well as monitor the efficacy of preventive and treatment strategies aimed to control periodontal diseases.[82–84]

The authors are aware that BOP score is merely a measure of the extent of gingival inflammation rather than a method to assess the severity of the inflammatory condition. The limitations arising from the use of semiquantitative indices, such as GI, to diagnose gingivitis patients have been addressed above. Although severity of gingival inflammation may be well defined on a site-specific basis,[35] signs of gingival inflammation, such as gingival volume and color changes (however assessed), can be hardly merged with BOP% at a patient-level, and they would eventually result in a subjective, time consuming and impractical procedure to establish a universally-acceptable GC definition.

Beyond the underlying tissue inflammation, there are patient factors that can affect the gingival response to mechanical stimulation by a probe. Previous studies have clearly shown that the individual tendency to develop gingival bleeding after probe stimulation may be a host-related trait that can depend on several patient-related factors.[6,77,191] Smoking has been consistently shown to suppress the gingival bleeding response during development of gingivitis,[89,225–228] while a limited number of studies have shown that under steady-state conditions smoking increases the likelihood of a gingival bleeding response to probing.[229,230] Patients on anticoagulant medications (e.g., aspirin) exhibit increased bleeding response to probing.[231–234] Among patients with similar ethnic background and plaque levels, differences in genetic background might also account for different BOP responses.[191,198,201] Despite evidence suggesting a greater susceptibility of thin gingival tissues to mechanical trauma,[235,236] the significance of gingival quality/dimensions (i.e., periodontal phenotype) for the BOP response remains unresolved.[230,237] Nevertheless, the presence of patient determinants known to affect the BOP response should be taken in consideration when determining the periodontal inflammatory conditions, in general, and when diagnosing a GC, in particular.

Definition of gingivitis in a patient with an intact periodontium

A patient with an intact periodontium is diagnosed as a GC as follows (Table 6): localized gingivitis, defined as a patient presenting with a BOP score ≥10% and ≤30%, without attachment loss and radiographic bone loss. This case may be associated with patient perception of bleeding gums, and a scarce, if any, impact on quality of life; or generalized gingivitis, defined as a patient presenting with a BOP score > 30%, without attachment loss and radiographic bone loss. This case

TABLE 6 Case definition of gingivitis in an intact periodontium

	Localized gingivitis	Generalized gingivitis
Probing attachment loss	No	No
Radiographic bone loss	No	No
BOP score	≥10%, ≤30%	>30%

is often associated with patient perception of bleeding gums, and a modest impact on quality of life.

A patient with a reduced periodontium[238] but without a history of periodontitis (e.g. gingival recession, crown lengthening) and a BOP score ≥10% would be diagnosed as a "GC on a reduced periodontium". A GC can also be graded as localized (BOP ≥10% and ≤30%) or generalized (BOP > 30%) (Table 7).

The same criteria may also be applied to a patient with a reduced periodontium[238] who has been successfully treated for periodontitis (periodontally stable patient), provided that no BOP positive sites show a probing depth ≥4 mm.

Both localized and generalized gingivitis should be managed by patient motivation, oral hygiene instruction, professional mechanical plaque removal, and implementation of self-performed mechanical plaque control, which may be supplemented by adjunctive use of antimicrobial/anti-inflammatory oral care products. Dietary advice and tobacco counseling are recommended when indicated.

The proposed GC diagnostic criteria would be of great value for defining and monitoring the disease in an epidemiological context, because such a GC definition should allow: 1) establishment of a framework that favors consistency of data interpretation across global epidemiological studies; 2) calculation of odds ratios and estimates of relative risk, both of which are sensitive to threshold definition, that are directly comparable between different studies; 3) assessment of the effectiveness of preventive measures and treatment regimens on a specific cohort of patients; 4) establishment of priorities for large-scale therapeutic actions/programs, with particular emphasis on their prognostic relevance (prevention of periodontitis) and impact on quality of life; and 5) undertaking of surveillance studies to monitor the prevalence and distribution of gingivitis consistently within a cohort as well as among different populations.[34]

However, it might be considered that in daily practice a patient with an intact periodontium or a reduced periodontium without history of periodontitis who shows even one site with clinical signs of gingival inflammation is worthy of professional intervention and, therefore, should be considered as a patient with sites of gingivitis.

A direct implication of the proposed GC definition is that a patient presenting with a BOP score < 10% without attachment loss and radiographic bone loss (intact periodontium) is considered clinically periodontally healthy. This definition is corroborated by previous studies where a BOP < 10% was used to define a periodontally-healthy case (Tables 3, 4, and 5).[153,158,208] Consistently, other reviews[6,35] from the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions reinforce the concept that a minimal level of gingival inflammation dispersed throughout the dentition can be considered as compatible with "clinical periodontal health". Hence, the ensuing issue is to identify which is the "minimal" amount of gingival inflammation within a dentition (i.e., a BOP score threshold) to distinguish a periodontally-healthy patient from a GC.[35] Some considerations support the use of minimal proportion of BOP+ sites as extent threshold in the definition of a GC: 1) the presence of a BOP < 10% is perceived as a clinically healthy condition by the patient;[91] 2) patients with a BOP score ≥15% have poorer quality of life compared to patients with BOP score < 15%;[93] and 3) a minimum extent threshold limits the possibility to categorize as GC those patients who present with a substantial transition of inflamed to healthy sites.[229]

For the patient with a reduced periodontium, without a history of periodontitis, or with successfully treated periodontitis (stable patient), the same criteria may be applied to define periodontal health, provided that no BOP positive sites show a probing depth ≥4 mm.

ACKNOWLEDGMENTS AND DISCLOSURES

TABLE 7 Case definition of gingivitis in a reduced periodontium without history of periodontitis

	Localized gingivitis	Generalized gingivitis
Probing attachment loss	Yes	Yes
Radiographic bone loss	Possible	Possible
Probing depth (all sites)	≤3 mm	≤3 mm
BOP score	≥10%, ≤30%	>30%

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