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Clinical Signs and Symptoms of Biofilm-Associated Infection in Chronic Wounds: A Systematic Review of Diagnostic Accuracy Studies

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ABSTRACT

The presence of biofilm in chronic wounds represents a major diagnostic challenge, as clinical manifestations are often subtle and laboratory confirmation remains limited. To identify clinical signs and symptoms (CSS) evaluated in validated tools or scales related to wound infection and biofilm, and to assess their diagnostic accuracy. A systematic review of diagnostic accuracy studies was conducted in accordance with PRISMA-DTA guidelines, searching six databases from inception to May 2025. Of 2064 records identified, four studies met inclusion criteria. All were focused on infection-related CSS; none were specifically designed to diagnose biofilm. Sensitivity and specificity varied substantially across CSS and study designs, and no validated, non-invasive diagnostic scale for biofilm was identified. The available evidence base is limited and heterogeneous. A preliminary list of candidate CSS is proposed to guide future validation studies and support earlier clinical recognition of biofilm-associated infection.

1 | Introduction

Within the wound healing process, inflammation is a critical phase in which wounds may stagnate and fail to progress [1]. A key local factor is microbial load. Whilst inflammation is an essential and physiological stage of wound healing, persistent microbial colonisation may exacerbate and dysregulate this phase, contributing to chronicity and delayed healing. The skin's own flora colonises the interior of wounds; however, chronic wounds may also be colonised by microorganisms originating from faecal or oral sources, particularly in hard-to-heal wounds, interacting with the wound bed. Infection has been defined as the condition in which microorganisms present in a lesion multiply to the point of eliciting a local, spreading, or systemic host response, thereby disrupting the physiological wound healing process [2]. The EWMA document on 'antimicrobials and non-healing wounds: an update' [3] proposes defining infection as 'the presence of clinical signs and symptoms of inflammation, which may be supported by various laboratory tests.' It is important to recognise, however, that clinical signs and symptoms of inflammation may differ substantially between acute and chronic wounds. In chronic wounds, inflammatory manifestations are often subtle or covert, frequently presenting as low-grade, persistent inflammation rather than the classical acute signs [2, 4]. This distinction is particularly relevant when considering biofilm-associated infection, where overt clinical features may be absent. Theoretically, the presence of microorganisms in the wound bed has been categorised within a continuum of

KEY POINTS

- Biofilm-Associated Infections represents a major diagnostic challenge with important implication for its management.
- There a not a non invasive point of care tool to diagnose biofilm at the bedside.
- A preliminary list of candidate clinical signs and symptoms is proposed to further validation and to support clinical practice.

infection [2]. Some authors [3] also describe a phase in which wounds fail to heal but may show significant microbial involvement without apparent clinical signs and symptoms (CSS), aside from low-grade inflammation, commonly referred to as biofilm. Although, in earlier investigations, the concept of biofilm may have been masked under the term 'critical colonisation', a term increasingly discouraged due to its imprecision and even recommended for removal from professional vocabulary [2, 5]. This concept also overlaps with that of 'infection', which may be caused by either planktonic (free-floating) or aggregated (biofilm-forming) microorganisms.

The role of biofilm in chronic and persistent infections has been extensively investigated over the past six decades. Early work by Costerton et al. [6] fundamentally changed the understanding of microbial behaviour in vivo, demonstrating that bacteria predominantly exist in structured, surface-attached communities rather than in planktonic form. This paradigm shift established biofilm as a central mechanism underlying chronicity and recalcitrance to treatment in a wide range of infections, including those affecting wounds. The application of this biofilm paradigm to chronic wound pathology has progressively evolved since the 1970s and 1980s, providing a biological rationale for the persistent low-grade inflammation and impaired healing observed in hard-to-heal wounds. In 2008, three revealing studies [7–9] were also published concerning the role of biofilm in wound healing.

The presence of biofilm in a wound results in low tissue oxygen tension and pH alkalinisation, which elevates levels of proteases and reactive oxygen species and increases cellular senescence [10]. Biofilm alone is rarely the sole cause of chronicity, but it significantly contributes to other potential factors that maintain the wound in a persistent, low-grade inflammatory phase [11, 12].

High-quality evidence [11, 13] has identified biofilm in approximately 80% of chronic wounds. If we accept the premise that biofilm is present in most chronic wounds, then it is necessary to know how to identify and treat it appropriately [14].

Microscopic identification of bacterial structural organisation is considered the gold standard (GS), achieved by confocal laser scanning microscopy and scanning electron microscopy [15]. In the absence of laboratory-confirmed diagnosis, best practises suggest that biofilm may be presumed in wounds that display CSS indicative of low-grade chronic inflammation [16]. However, typical clinical signs of local infection may not manifest as expected in the presence of certain pathologies [17]. Additionally, it is not yet clear how many CSS must be observed to confirm the presence of infection or biofilm. In 2014 and 2015, Metcalf et al. [18] and Percival et al. [19] published algorithms for diagnosing biofilm based on wound evolution in response to interventions. Tools such as that of Dissemond et al. [12] detect local infection based on CSS, although they do not distinguish between planktonic and biofilm phenotypes of the causative agents. Metcalf et al. [18] also proposed a differential approach to distinguish biofilm (a viable, structured bacterial community) from slough (non-viable host-derived devitalised tissue), which frequently causes diagnostic confusion [18, 20]. The reliability of macroscopic CSS has been questioned due to instances in which biofilm is found without overt symptoms [10, 21]. Consequently, several scientific societies have proposed the existence of subtle CSS indicative of local infection [10, 16, 20, 22].

Currently, it is unclear whether biofilm alone, with or without associated clinical signs, always leads to delayed wound healing or whether infection, regardless of biofilm, is the primary concern [3]. In any case, early diagnosis of infection and/or biofilm in chronic wounds is essential, as delayed identification may hinder healing by failing to address one of the underlying causes of stagnation [23].

Due to the lack of consensus on this issue, we posed the following research question: What clinical signs and/or symptoms are used on validated scales or tools for wound infection and biofilm? Our objective was to review the literature and, if feasible, establish a set of CSS that could help identify suspected biofilm in chronic wounds. We hypothesised that no validated scale currently exists that can non-invasively assess the presence or absence of biofilm in chronic wounds based solely on in situ observation of CSS.

2 | Materials and Methods

A systematic review of diagnostic accuracy studies was conducted from June 2023 to August 2023, with a replication of the search in May 2025. The review was based on a protocol registered in PROSPERO (CRD42023428044) [24]. The manuscript was prepared according to the PRISMA-DTA 2018 guidelines [25].

2.1 | Eligibility Criteria

The following criteria were applied for literature selection:

Inclusion criteria:

- Papers concerning infection and biofilm characteristics in chronic wounds.
- Documents reporting on the validity or reliability of CSS for infection and/or biofilm in chronic wounds
- Studies using microscopy, fluorescence, molecular analysis, or cultures as the gold standard (GS).
- Studies published in Spanish, Portuguese, or English, considering the authors' language proficiency
- No time restriction was applied in the search.

Exclusion criteria:

- Studies focused on laboratory instruments rather than CSS.

2.2 | Information Sources and Search Strategy

Documents were retrieved from the Web of Science Core Collection, MEDLINE (via PubMed), CINAHL, Scopus, the Cochrane Library, and the Virtual Health Library. Additional articles were identified through reverse citation searching and primary source tracing.

Searches were performed from June 1st to August 30th, 2023 (and repeated in May 2025 to identify any new articles). The keywords used included: biofilm, infection, wound, skin ulcer, chronic wound, index, scale, score, algorithm, tool, assessment, validity, accuracy, reliability, management, symptom, sign, and related truncations. The search strategy was intentionally focused on terms related to diagnostic tools or scales assessing infection and biofilm-related clinical signs and symptoms. Broader mechanistic terms such as 'inflammation' were not included as isolated keywords, as the objective was not to explore inflammatory pathways but to identify validated clinical diagnostic instruments. The terms 'in vitro,' 'ex vivo,' 'model,' 'surgical,' 'burn,' and their derivatives were excluded. The search was filtered by language (English, Spanish, and Portuguese), but no time limits were applied. The search formulas are shown in Table 1 for MEDLINE and were adapted for the other databases.

TABLE 1 | Search strategy formulas.

#1	((‘chronic wound’ OR ‘skin ulcer’) AND (infection OR biofilm)) AND (index OR score OR scale OR tool OR assessment OR algorithm) AND (validity OR reliability OR accuracy)
#2	‘wound biofilm’[All Fields] AND (‘sign*’[All Fields] OR ‘symptom*’[All Fields] OR ‘clinical evaluation*’ [All Fields] OR ‘clinical assessment*’ [All Fields])
#3	(‘wound’ [Title/Abstract] OR ‘skin ulcer’ [Title/Abstract]) AND (‘infection’ [Title/Abstract] OR ‘biofilm’ [Title/Abstract]) AND (‘index’ [Title/Abstract] OR ‘score’ [Title/Abstract] OR ‘scale’ [Title/Abstract] OR ‘tool’ [Title/Abstract] OR ‘assessment’ [Title/Abstract] OR ‘algorithm’ [Title/Abstract]) AND (‘validity’ [Title/Abstract] OR ‘reliability’ [Title/Abstract] OR ‘accuracy’ [Title/Abstract]) NOT ‘surg*’ [Title/Abstract]
#4	((‘chronic wound’ [Title/Abstract] OR ‘skin ulcer’ [Title/Abstract]) AND (infection[Title/Abstract] OR biofilm[Title/Abstract])) AND (index[Title/Abstract] OR score[Title/Abstract] OR scale[Title/Abstract] OR tool[Title/Abstract] OR assessment[Title/Abstract] OR algorithm[Title/Abstract]) NOT surgical[Title/Abstract] NOT burn*[Title/Abstract] NOT ‘in vitro’ [Title/Abstract] NOT ‘EX VIVO’ [Title/Abstract]
#5	‘chronic wound infection’ [Title/Abstract] AND (‘sign*’ [Title/Abstract] OR ‘symptom*’ [Title/Abstract]) NOT ‘treat*’ NOT ‘managem*’ NOT ‘model’
#6	‘wound biofilm’ [Title/Abstract] AND (‘sign*’ [Title/Abstract] OR ‘symptom*’ [Title/Abstract]) NOT ‘treat*’ NOT ‘managem*’ NOT ‘model’

Note: Adapted to PubMed syntax.
Source: Authors' own work.

2.3 | Study Selection Process

Article selection was performed independently by two researchers. The search strategies were replicated across all databases. Inter-rater agreement was assessed after an initial sample (from the MEDLINE search via PubMed). Subsequently, articles retrieved from the remaining databases were compared, and a third reviewer was consulted in case of discrepancies.

Once articles were selected, duplicates were automatically removed using Mendeley v1.19.8.

2.4 | Data Extraction Process

Two ad-hoc tables were created for data extraction. The first table included: year, author, CSS, population and sample, GS, infection vs. non-infection outcomes, and methodological quality. The second table reported the sensitivity and specificity of each CSS compared to the gold standard used. If sensitivity and specificity data were not broken down by individual CSS, grouped data were summarised narratively.

2.5 | Risk of Bias Assessment and Methodological Quality of Individual Studies

To assess the methodological quality of the included studies, the STARD 2015 guidelines [26] were applied. Each item of the STARD checklist specifies essential components for diagnostic accuracy studies. Each fulfilled item received one point. The total number of fulfilled items was then divided by the number of checklist items and multiplied by 100 to obtain a percentage score.

The following quality scale was applied:

- 0%–25%: Very poor quality or very high risk of bias
- 26%–50%: Poor quality or high risk of bias
- 51%–75%: Good quality or low risk of bias
- 76%–100%: Very high quality or very low risk of bias

Although our protocol established a minimum quality threshold of 60% to ensure adequate methodological rigour, we ultimately decided to include all selected studies due to the limited number of available diagnostic accuracy publications.

2.6 | Analysis and Synthesis of Results

Due to the lack of raw data in the primary studies, only a descriptive narrative synthesis was conducted.

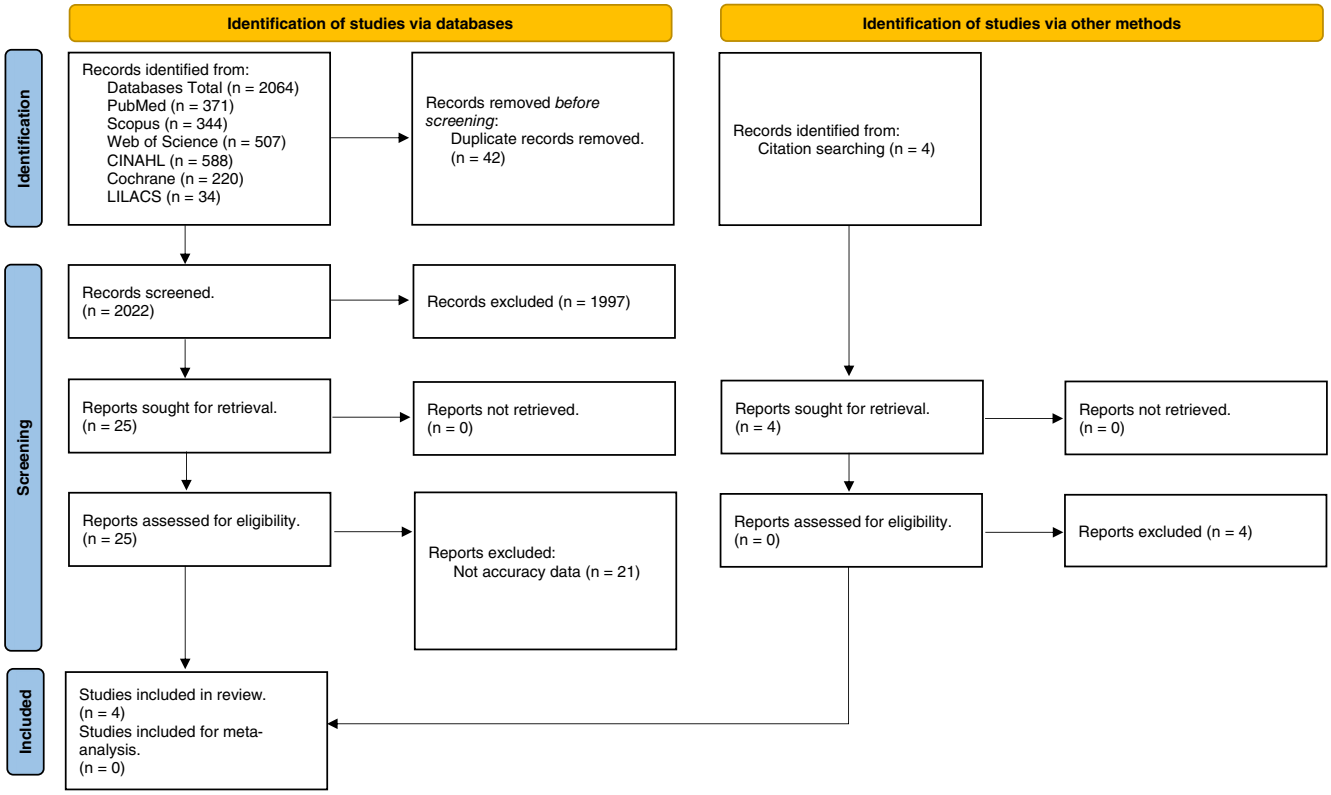

FIGURE 1 | PRISMA flow diagram on study selection.

3 | Results

A total of 2064 articles were identified. After screening titles, 130 were selected. Forty-two duplicates were removed. Eighty-eight abstracts were reviewed, and 67 were excluded for not meeting eligibility criteria, leaving 25 articles for full-text review. An additional 4 references were identified through reverse citation search during the reading process. Ultimately, 4 studies were included in the final analysis (Figure 1). The additional search conducted in May 2025 did not yield any new articles.

Based on the STARD tool [26], only the study by Le et al. [27] scored above 60%, indicating the overall low methodological quality of diagnostic accuracy studies in wound research. Nonetheless, since only four articles [27–30] were retrieved, all were included in this review, with their respective quality scores reported in Table 2.

Table 2 also shows the wide variability in wound types included in the studies, with a predominance of diabetic foot ulcers (DFUs), leg ulcers (LUs), venous ulcers (VUs), and pressure injuries (PIs). Regarding the gold standard (GS), Gardner et al. [28] examined the relationship between the clinical identification of CSS as described in the literature at the time and quantitative culture. The reliability and validity tests of the NERDS and STONEES tool by Woo et al. [29] were performed against semi-quantitative culture. Armstrong et al. [30] conducted a study on a specific population from the FLAAG study [27]. Both Le et al. [27] and Armstrong et al. [30] compared CSS to diagnostic results using immunofluorescence and biopsy microscopy techniques.

Table 3 presents the sensitivity and specificity values for the CSS with available data. Gardner et al. [28] evaluated classical signs (pain, erythema, oedema, heat, and purulence) and secondary signs typical of chronic wounds (serous exudate, delayed healing, friable granulation tissue, discolouration of granulation tissue, and malodour), based on the classic CSS infection list and the subtle infection signs described in 1994 by Cutting et al. [33]. Delayed healing and discoloured granulation tissue had the highest sensitivity (0.81 and 0.82, respectively). Increased temperature (0.18), pain (0.36), and malodour (0.36) showed the lowest sensitivity. Increased pain [1], delayed healing [1], and malodour (0.88) showed the highest specificity. Discoloured granulation tissue (0.56), delayed healing (0.64), and purulent exudate (0.64) were the least specific. Regarding positive predictive value, increased pain had a value of 1, followed by friable granulation tissue and malodour. These were the only signs exceeding a value of 0.5.

It was also observed that two factors correlated with confirmed infection: low tissue oxygen pressure, assessed non-invasively using transcutaneous oxygen monitoring ($TcPO_2$) and the presence of necrotic tissue. The study supports the use of this set of clinical signs for active infection screening, whilst noting that signs based on touch, colour, or smell are heavily influenced by observer subjectivity.

Woo et al. [29] conducted diagnostic accuracy testing on the NERDS and STONEES tool developed by Sibbald et al. [34]. This tool classifies signs into superficial infection or colonisation (NERDS) and deep infection (STONEES). The highest sensitivity was observed for oedema (0.87), increased temperature (0.76), and increased exudate (0.70). The lowest sensitivity

TABLE 2 | Characteristics of the studies included in the review.

Author, year	CSS	Population/Sample	Gold standard results (infected/non-infected)	Methodological quality (STARD)
Gardner et al. [26]	Increased local pain; Erythema; Edema; Warmth; Purulent exudate; Serous exudate concurrent with inflammation; Delayed healing; Discoloration of granulation tissue; Friable granulation tissue; Tunnelling or cavities in granulation tissue; Malodour; Worsening	$n = 36$ PI [19], VU [7], SS [6], CT [2], DFU [2]	Quantitative biopsy culture Growth $> 10^4$ CFU/g (Infected [11], Non-infected [25])	55.9% Good or low risk
Woo et al. [27]	NERDS	$n = 112$	Semi-quantitative swab culture	44.1%
	Stalled wound; Exudative wound; Red and bleeding granulation tissue; Tissue discoloration; Malodour or onset of odour.	LU [31], DFU (68)	Growth $> 10^4$ CFU/g (NAD)	Poor or high risk
	STONEES			
	Increased size; Increased temperature; Probe to Bone +; Worsening; Exudate; Erythema and edema; Odour.			
Le et al. [25]	Subtle signs of local infection	$n = 350$	Quantitative biopsy culture	70.6%
	Hypergranulation; Friable granulation tissue; Bridging or undermining in granulation tissue; Worsening; Delayed healing; New or increasing pain; Increased malodour.			
	Visible signs of local infection		Growth $> 10^4$ CFU/g	
	Erythema; Local warmth; Edema; Purulent exudate; Delayed healing; New or increasing pain; Increased malodour.	DFU (138), PI [22], VU (106), SS (60), Other [24]		Good or low risk
	Spreading infection		Infected (287), Non-infected (63)	
	Extension of erythema or edema; Lymphangitis; Crepitus; Worsening; Malaise, lethargy or general nonspecific decline; Appetite loss.			
Armstrong et al. [28]	Mild infection	$n = 138$	Quantitative biopsy culture	41.2%
	Local induration, Erythema < 2 cm from wound; Local tenderness or pain; Purulent exudate.			
	Moderate infection		Growth $> 10^4$ CFU/g	
	Erythema ≥ 2 cm from wound margin and/or involvement of deeper tissues (tendon, joint, bone (Probe to Bone +), or muscle).	DFU (138)		Poor or high risk
	Severe infection		Infected (123), Non-infected [15]	
	Diabetic foot infection with 2 or more of: Temperature > 38°C or < 36°C; HR > 90 bpm; RR > 20 or PaCO2 < 32 mmHg; Leukocytes $> 12000/mm^3$ or $> 10\%$ band forms.			

Abbreviations: CSS, Clinical Signs and symptoms; CT, Chronic traumatic; DFU, Diabetic foot ulcer; LU, Leg ulcer; NAD, No available data; PI, Pressure injury; SC, Secondary incision; SS, Surgical site; VU, Venous ulcer.
Source: Authors' own work.

TABLE 3 | Diagnostic accuracy (sensitivity and specificity) of individual signs and symptoms indicating high microbial load, as proposed in the included studies.

	Gardner et al. [26] (CSS by Cutting et al. [29])		Woo et al. [27] (CSS by NERDS & STONEES [30])		Le et al. [25] (CSS by IWGDF [32])		Amstrong et al. [28] (CSS by IWII [33])	
CSS	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
Delayed healing	0.81	0.64	0.32	0.47	NAD	NAD	*NAD*	NAD
Increased pain	0.36	1	NAD	NAD	NAD	NAD	NAD	NAD
Local erythema	0.55	0.68	0.87	0.44	NAD	NAD	NAD	NAD
Oedema	0.64	0.72	0.87	0.44	NAD	NAD	NAD	NAD
Heat	0.18	0.84	0.76	0.71	NAD	NAD	NAD	NAD
Purulent exudate	0.18	0.64	NAD	NAD	NAD	NAD	NAD	NAD
Inflammation and serous exudate	0.55	0.72	0.70	0.64	NAD	NAD	NAD	NAD
Pale tissue	0.64	0.56	0.62	0.78	NAD	NAD	NAD	NAD
Friable granulation	0.82	0.76	0.45	0.86	NAD	NAD	NAD	NAD
Pocketing	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Malodour	0.36	0.88	0.37	0.86	NAD	NAD	NAD	NAD
Worsening	0.46	1	0.37	0.89	NAD	NAD	NAD	NAD
Wound size increase	NAD	NAD	0.50	0.83	NAD	NAD	NAD	NAD
Probe to bone+	NAD	NAD	0.40	0.81	NAD	NAD	NAD	NAD
Hypergranulation	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Local induration	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Oedema extension	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Erythema extension	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Crepitation	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Lymphangitis	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Discomfort, lethargy or general deterioration	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Loss of appetite	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Erythema extension with involvement of tendon, joint, bone or muscle	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
>=2 local signs plus Temp>38°C or<36°C, HR>90 bpm or RR>20	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

Abbreviation: NAD, no available data.
Source: Authors' own work.

was noted for non-healing wounds (0.32), malodour (0.37), and Probe to Bone + (0.40). The most specific signs were new delayed healing (0.89), malodour (0.86), and friable granulation tissue (0.86). The least specific signs included erythema and oedema (0.44), non-healing wounds (0.47), and increased exudate (0.63).

It was found that pallor of the tissue, increased exudate, and friable granulation tissue increased the likelihood of positive infection diagnosis by fivefold. Elevated temperature was associated with an eightfold increase in likelihood.

In the Fluorescence Imaging Assessment and Guidance (FLAAG) study, Le et al. [27] compared the diagnostic accuracy of the MolecuLight fluorescence imaging device (which detects microbial loads $>10^5$ CFU/g) with infection-related clinical signs, using quantitative culture by biopsy as the reference standard. The study first compared clinical signs plus fluorescence against culture results, and then clinical signs alone against culture. If the evaluators identified three or more criteria from the IWII (2016) list [32], or a clear sign of infection, they recorded a positive clinical infection diagnosis.

Due to the clinical and contextual heterogeneity of the studies, as well as the lack of raw data for each individual CSS, a meta-analysis was not feasible. However, it was possible to compile a list of all CSS described across the four studies. Therefore, our working hypothesis is that the CSS listed by primary studies, in Table 3, should be considered when evaluating suspected biofilm-related infection. These signs show the best sensitivity and specificity for diagnosing infection, though further investigation is needed to determine whether they are equally effective in diagnosing biofilm.

4 | Discussion

None of the studies included in this review were specifically designed to evaluate the diagnostic accuracy of CSS for the diagnosis or suspicion of biofilm in chronic wounds. All of them focused on validating individual or combined sets of CSS for infection diagnosis. Nonetheless, the currently proposed CSS are primarily derived from expert consensus, which represents the lowest level of evidence [35], and these studies aim to validate such signs to strengthen their evidentiary basis. Additionally, a recent work by Hurlow and Bowler [4] provides a clinically oriented distinction between acute and chronic (biofilm-associated) wound infection, emphasizing differences in microbiological phenotype, inflammatory profile and clinical presentation. Despite this conceptual clarification, no validated diagnostic accuracy tool based solely on clinical signs currently exists to identify biofilm in chronic wounds.

Only two studies in this review assessed the diagnostic accuracy of individual CSS [28, 29]. The other two studies [27, 30], which actually share a common data source, evaluated the performance of combined CSS sets (one from the IWGDF and the other from the IWII) against quantitative cultures and an alternative diagnostic method (fluorescence imaging).

According to the reviewed literature, Gardner et al. [28] were the first to establish a validation list of CSS based on the work of Cutting et al. [33]. Later proposals, such as those from IWII [2] and WUWHS [5], were based on these initial lists. In the case of Armstrong et al. [30], given that the study focused solely on patients with diabetic foot ulcers, the IWGDF [36] classification was applied. This classification is supported by earlier research on such patient populations [36–38].

The CSS included in each individual study are summarised in Table 2, and as shown in Table 3, not all studies provided individual-level diagnostic accuracy data. Gardner et al. [28] and Woo et al. [29] did, but a meta-analysis was not feasible since Gardner provided raw data [28], whilst Woo only reported calculated metrics [29], in addition to clinical heterogeneity and differences in GS methodology. Le et al. [27] and Armstrong et al. [30] analysed CSS in aggregate rather than individually.

As mentioned earlier, all the described CSS have been associated with infection, regardless of whether microorganisms are present in planktonic or biofilm forms. Another complication is that many of the listed CSS are not pathognomonic for infection. This necessitates a comprehensive assessment of the wound to enable differential diagnosis. Some wounds may exhibit inflammatory signs not due to infection, but rather due to their aetiology or underlying pathophysiology [2, 22]. Others may show signs of low-grade inflammation related to microbial activity that are not easily recognised by all clinicians. This remains a major challenge today [2, 22].

As an example, some signs like hypergranulation are not exclusive to inflammatory processes. Hypergranulation may result from a high microbial load, but also from hypoxia due to occlusive dressings, friction, excessive moisture, foreign body reactions, or may even resemble malignancy in the tissue [39–41].

Another controversial issue is the role of laboratory testing in infection diagnosis. Although culture techniques remain in use, they present recognised limitations, including turnaround time, sampling variability and limited ability to reflect polymicrobial biofilm communities [15]. Across the included studies, reference standards varied (quantitative biopsy, semiquantitative swab, fluorescence imaging), contributing to methodological heterogeneity and limiting comparability. Importantly, none of the studies used a gold standard specifically designed to detect biofilm.

In relation to the studies included in this review: Gardner et al. [28] collected biopsies after cleansing the wound with normal saline (NS), avoiding necrotic tissue. Woo et al. [29] used the Levine swabbing technique after NS cleansing. Le et al. [27] performed up to three biopsies at different points within the wound under local anaesthesia, targeting the centre of the lesion and areas with fluorescence or positive clinical signs. Armstrong et al. [30] conducted a post hoc analysis of a defined population from the Le et al. [27] study, using the same biopsy methodology. However, some methodological heterogeneity is evident across studies. Notably, none of the studies employed techniques specifically intended to detect suspected biofilm.

Additionally, a recent study [42] identified a broad spectrum of diagnostic approaches and highlighted that diagnostic accuracy improves significantly when microbiological analyses

are combined with clinical assessments. However, the heterogeneity and methodological variability across studies hinder meta-analysis. The authors suggested that future research should focus on standardised and homogeneous study designs to improve the assessment of diagnostic accuracy of alternative methods. In parallel, Edwards et al. [43] conducted a similar systematic review focused on diagnostic accuracy studies for detecting infection in chronic wounds. However, that review focused on instrumental techniques such as fluorescence imaging and enzymatic analysis of wound exudate, rather than on CSS.

Returning to the central theme of this paper, the CSS, Vestjens et al. [44] concluded that variability in CSS identification is reduced when a checklist-guided assessment is used instead of open-ended questioning. This supports our team's interest in developing and validating a paper-based checklist tool for identifying CSS consistent with biofilm-related infection.

Along these lines, a recent scoping review by Ivory et al. [31] and an e-Delphi study protocol [45] and the recent final paper from the e-Delphi study [46] have been published to determine the signs, symptoms, and biomarkers associated with biofilm in chronic wounds. Ivory et al. [31] identified potential CSS and biomarkers consistent with biofilm presence, whereas our review focused exclusively on identifying CSS supported by sensitivity and specificity data.

Ivory et al. [31], despite including texts from as early as 2001, provide valuable consensus work; however, conceptual overlap between 'biofilm', 'critical colonisation' and 'infection' remains challenging in the literature, an aspect we consider a significant limitation in their work.

Of the four studies selected in our review, only the articles by Armstrong et al. [30] and Le et al. [27] were considered in the work by Ivory et al. [31], but both were excluded due to 'no concept data.' However, all the CSS analysed in our review are included in the list of CSS compatible with biofilm proposed by Ivory et al. [31].

In our own analysis, we did not identify any validated scales for the detection of biofilm in chronic wounds. The closest alternatives are unvalidated diagnostic algorithms [18, 19]. Ivory et al. [31] propose 26 CSS or sets of CSS and biomarkers, but they lack the necessary rigour for accurate biofilm diagnosis. After their Delphi exercise [46], they found that in the opinion of an international panel of experienced wound care clinicians, there are 11 CSS that are very likely to indicate the presence of biofilm in chronic wounds but they remark that, as previously observed, little validation work has been done in this area, and they advocated that efforts should be made to validate reported signs and symptoms of biofilm in chronic wounds.

Importantly, the absence of validated diagnostic scales for biofilm should not be interpreted as questioning the clinical relevance of biofilm in chronic wound pathology. On the contrary, the biofilm paradigm is strongly supported by decades of microbiological and translational research, with substantial evidence demonstrating its role in persistent inflammation, antimicrobial tolerance, and delayed healing. Rather, our findings highlight a translational gap between well-established biological knowledge and the availability of validated, clinically applicable diagnostic tools.

Both research teams (Ivory et al. and us) agree on the importance of focusing efforts on validating these CSS to identify the presence of biofilm in chronic wounds. For this reason, we have already established contact to collaborate and ensure that future results are promising, multidisciplinary, and geographically broad in scope.

4.1 | Limitations

In addition to the possible limitations related to the GS used in the included studies and its implications for accurate infection/biofilm diagnosis, this review may present some inherent limitations typical of systematic reviews:

- Some relevant studies may not have been retrieved due to the search strategy or databases used. However, we made an exhaustive effort to collect as much information as possible.
- We only included studies published in English, Spanish, or Portuguese. Nevertheless, no studies were identified and excluded based on language
- The methodological quality of most studies was below 60% of STARD items, which may imply a potential risk of bias in the individual studies analysed. As previously stated, we chose to retain these studies due to the limited number of diagnostic accuracy articles available
- Two of the included diagnostic accuracy studies predate the widespread adoption of the biofilm paradigm in wound research. Consequently, biofilm was not explicitly conceptualised in their design, which may limit the direct applicability of their findings to contemporary biofilm-focused frameworks.
- It was not possible to conduct a meta-analysis of the included studies. The main reasons were the clinical heterogeneity amongst them and the lack of raw data necessary for such analysis.

5 | Conclusions

It was not possible to answer the question: 'What clinical signs and/or symptoms are used on validated scales or tools for wound infection and biofilm?' because, to date, no such validated scale exists in the literature.

The identified diagnostic accuracy studies are of limited quality and focus on CSS used for infection diagnosis, based on quantitative and/or semi-quantitative culture techniques, which themselves have known limitations.

These findings support the urgent need for standardised diagnostic accuracy studies to enable earlier recognition and improved management of biofilm-associated infection. To guide this forward phase, a proposed 'Core Clinical Signs and Symptoms list' is presented:

Proposed Core Clinical Signs and Symptoms (CSS):

Based on consistency across included diagnostic accuracy studies and alignment with recent international consensus work [45], the following indicators emerge as the most consistently supported candidates for biofilm-associated infection in chronic wounds. Although altered wound pH and reduced tissue oxygenation have been associated with biofilm presence in experimental and translational studies, these parameters require instrumental measurement and were not consistently evaluated within diagnostic accuracy studies based on clinical observation. Therefore, they were not included within the proposed clinical signs and symptoms framework:

Core (most consistently supported) indicators:

1. Persistent shiny/slimy wound surface layer that reforms rapidly after debridement.
2. Failure to respond as expected to antimicrobial therapy.
3. Delayed healing or stalled wound despite optimal management.
4. Infection persisting > 30 days or recurrent (waxing and waning) infection.
5. Poor quality granulation tissue (friable, discoloured, fragile, or hypergranulation).
6. Persistent or prolonged low-grade inflammation.
7. Wound duration > 6 weeks.
8. Soft tissue deterioration despite appropriate therapy.
9. Signs of local infection (e.g., erythema, oedema, serous exudate, new breakdown, pain).
10. Tunnelling and/or undermining.
11. Presence of slough or fibrin.

Additional candidate indicators (less consistently supported):

The following indicators were reported in the literature but did not demonstrate consistent diagnostic accuracy data or consensus support:

- Increased exudate.
- Malodour.
- Low-grade fever.
- Isolated wound pain.
- Pale edematous wound bed.
- Negative cultures despite clinical suspicion.
- Systemic antibiotic history.
- General patient deterioration.
- Sepsis (unlikely to represent a local biofilm-specific marker).

This categorisation is exploratory and intended to synthesise current evidence rather than to represent a validated diagnostic tool.

In our next research phase, we will begin by assessing content validity using a consensus technique with experts. This will be followed by clinical validation, comparing the presence of these CSS with results from fluorescence at point of care, confocal laser microscopy, molecular techniques, and expert clinical judgement. Additionally, it is essential to establish a threshold to determine the presence and/or infection caused by biofilm. In other words, how many concomitant CSS are required to define the presence or infection of biofilm in a wound?

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Conflicts of Interest

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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