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Review Article

Assessing pain objectively: the use of physiological markers

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Summary

Pain diagnosis and management would benefit from the development of objective markers of nociception and pain. Current research addressing this issue has focused on five main strategies, each with its own advantages and disadvantages. These encompass: (i) monitoring changes in the autonomic nervous system; (ii) biopotentials; (iii) neuroimaging; (iv) biological (bio-) markers; and (v) composite algorithms. Although each strategy has shown areas of promise, there are currently no validated objective markers of nociception or pain that can be recommended for clinical use. This article introduces the most important developments in the field and highlights shortcomings, with the aim of allowing the reader to make informed decisions about what trends to watch in the future.

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Background

Pain is fundamental to human existence. It has shaped our evolution, and aids our ability to avoid dangerous hazards. Nevertheless, striving to alleviate such suffering is at the heart of medicine, and one of the anaesthetist's essential roles.

The key to adequate pain management is assessing its presence and severity, identifying those who require intervention and appreciating treatment efficacy. The experience of pain is complex, as reflected by its definition as "*an unpleasant sensory and emotional experience, associated with actual or potential tissue damage*" [1]. Pain therefore relates to both the noxious input via peripheral nerves and central modulation integrating different modalities such as affect, experience or personality. Current 'gold-standard' pain assessment tools rely on self-reporting, requiring an individual both to process external information and to communicate this personal experience [2]. Circumstances exist where this is not possible, or where it is unreliable. In these situations, surrogate markers utilise changes in behavioural or physiological parameters [3, 4]. However, their use can be associated with considerable shortcomings. They may be unreliable [5], hampered by observational bias, or influenced by disease processes or pharmacological interventions. Developing an objective method of pain assessment therefore needs to ensure tools that are sensitive and specific to pain. They need to be observer-independent, not reliant on the patient's ability to communicate and not influenced by disease characteristics. This article reviews evidence available on the most promising current approaches, and highlights areas for possible future developments.

Methods

A literature search of the electronic database PubMed (see www.ncbi.nlm.nih.gov/pubmed/) was undertaken using the following keywords, individually and in combination: "pain"; "nociception"; "heart rate variability"; "analgesia nociception index"; "cardiorespiratory coherence algorithm"; "cardiovascular depth of analgesia"; "surgical plethysmographic index"; "surgical stress index"; "fluctuations of skin conduction"; "pupillometry"; "nociception flexion reflex"; "evoked potentials"; "positron emission tomography"; "magnetic resonance imaging"; "functional near infrared spectroscopy"; "electro-encephalography"; "magneto-encephalography"; "bispectral index"; "composite variability index"; "entropy"; "biomarkers"; "stress hormones"; "markers of metabolism"; "markers of inflammation"; "cytokines"; "free radicals"; and "noxious stimulation response index". Articles identified from the above search, and published in English before May 2014, were reviewed. Publications were further screened for additional references regarding human clinical trials fitting the search criteria [6].

Results

Our review identified five main strategies for the development of objective measures of pain. These utilise: (i) changes in the autonomic nervous system; (ii) biopotentials; (iii) neuroimaging; (iv) biological (bio-) markers; and (v) composite algorithms.

Autonomic nervous system changes for pain assessment

Pain is thought to exacerbate the autonomic response to stress [7], a rationale supported by evidence showing a neuroanatomical overlap between nociceptive and autonomic pathways [8], increases in circulating stress hormones in response to pain [9], and by studies investigating the effect of postoperative analgesia on autonomic responses [10–13]. A number of potentially objective assessment tools have been developed that utilise the assumption that pain induces alterations in the autonomic nervous system. These include methods observing derived cardiovascular and respiratory parameters (heart rate variability, patterns of blood pressure and heart rate responses, pulse wave amplitude and pulse beat interval), skin sweating and pupillary changes (Table 1).

Heart rate variability

Interactions between the sympathetic and parasympathetic nervous system can be detected using computationally traceable measures of heart rate variability [41]. Changes to time and frequency analysis of intervals between consecutive heartbeats reflect autonomic reactivity to noxious stimulation [18, 42]. This easy-to-measure, non-invasive and real-time variable uses standard ECG monitoring, and can be used in both awake and sedated patients [43, 44]. However, heart rate variability can be influenced by numerous physiological and psychological conditions, such as age [45, 46], sex [47], co-morbidities [48–51], depth of anaesthesia [52], surgical stimulation [53], medications [54] and emotions [55]. Fluctuations in heart rate during breathing cycles (respiratory sinus arrhythmia) have been integrated to improve parameter accuracy [56]. It is thought to be influenced by noxious stimuli, particularly under steady-state anaesthesia, but translation into non-anaesthetised patients is contentious, along with the correlation between heart rate variability and pain intensity [16, 17]. Both pre-clinical studies and recent clinical trials suggest it could be developed in future into an objective pain assessment tool [14, 15, 24].

To correct for possible confounding factors, a number of real-time algorithms have been developed to evaluate heart rate variability in the setting of pain. These include:

- real-time Fourier high/low frequency ratios: although not specific to nociception, they are widely accepted methods for analysis of heart rate variability [14];
- the analgesia nociception index: this method combines electrocardiography and respiratory rate together with high-frequency heart rate variability, in a frequency domain analysis [57];
- the cardiorespiratory coherence algorithm: this analyses the coupling between heart rate and respiratory sinus arrhythmia patterns [25].

Experimentally, the analgesia nociception index shows an inverse linear relationship with both numerical rating and visual analogue scores [22, 24, 58]; however,

Table 1 Autonomic nervous system markers used in the assessment of pain.

Marker	Tool	Key findings	References
Heart rate variability (HRV)	Real-time Fourier low/high frequency ratio (LF/HF ratio)	*Intra-operative anaesthesia*	
		Correlation with haemodynamic responses	Jeanne et al. [14]
		Change in response to inadequate analgesia	Jeanne et al. [15]
		Postoperative	
		Correlation with pain scores	Chang et al. [16]
		Awake healthy volunteers	
		No correlation with pain intensity	Meeuse et al. [17]
		Responds to nociceptive stimulation	Koenig et al. (Review) [18]
	Analgesia nociception index (ANI)	*Intra-operative anaesthesia*	
		More sensitive than haemodynamic responses to noxious stimuli	Jeanne et al. [19]
		Correlation with noxious stimuli	Gruenewald et al. [20]
		Reflects different levels of noxious stimulation	Ledowski et al. [21]
		Postoperative	
		Correlation with NRS post-TIVA anaesthesia	Boselli et al. [22]
		No association with NRS after sevoflurane anaesthesia	Ledowski et al. [23]
		ANI immediately before extubation associated with postoperative pain intensity	Boselli et al. [24]
	Cardiorespiratory coherence algorithm	*Intra-operative anaesthesia*	
		Responds to noxious stimuli and anaesthetic bolus	Brouse et al. [25]
Heart rate and blood pressure changes	CARdiovascular DEpth of ANalgesia (CARDEAN) index	*Intra-operative anaesthesia*	
		CARDEAN-guided opioid administration resulted in reduced movement during colonoscopy	Martinez et al. [26]
		Correlation with noxious stimuli	Rossi et al. [27]
Peripheral pulsatile component of cardiac cycle	Surgical plethysmographic index (SPI)	*Intra-operative anaesthesia*	
		Responds to noxious stimuli and anaesthetic bolus (TIVA)	Huiku et al. [28]
		No association with nociception during spinal anaesthesia in awake patients	Ilies et al. [29]
		SPI-guided remifentanil administration resulted in reduced opioid consumption and faster recovery	Bergmann et al. [30]
		Postoperative	
		Moderate sensitivity and specificity to discriminate between low, moderate and severe pain; correlation with total opioid consumption	Thee et al. [31]
Electrodermal activity	Fluctuations of skin conductance (NFSC)	Detection of nociception and pain	Storm (review) [32]
		Postoperative	
		Correlation with pain scores (adults)	Ledowski et al. [33]
		Accurate prediction of absence of moderate to severe pain (children)	Hullett et al. [34]
		Weak correlation with pain scores (children)	Choo et al. [35]
		Awake healthy volunteers	
		Correlation with individual heat evoked pain intensity, but high variability between individuals	Loggia et al. [36]
Pupil reflexes	Pupillometry	*Labour pain*	
	PD (pupil diameter)	Correlation with PD and PLRA	Guglielminotti et al. [37]
	PDR (pupillary dilatation reflex)	*Intensive care*	
	PLRA (pupillary light reflex amplitude)	PD variation to tetanic stimulation predicted insufficient analgesia during tracheal suctioning	Paulus et al. [38]
		Postoperative	
		Correlation of PDR with VRS	Aissou et al. [39]
		No association of PD or PLRA with NRS	Kantor et al. [40]

NRS, numerical rating scale; TIVA, total intravenous anaesthesia; VRS, verbal rating scale.

its accuracy in postoperative pain detection has been inconclusive [23]. Both the analgesia nociception index and the cardiorespiratory coherence algorithm reflect noxious stimulation levels, and respond to increasing plasma concentration and boluses of opioids [19–21, 59–61]. These findings may nevertheless be influenced by the mode of anaesthesia and residual effects in the postoperative period. Despite this method's offering easy practical applications, it remains unclear whether the complex computational algorithms are specific enough to nociception or pain in a clinical setting.

CARdiovascular DEpth of ANalgesia index

Noxious stimulation induces minor increases in blood pressure, followed by an increase in heart rate. The CARdiovascular DEpth of ANalgesia (CARDEAN) index was developed to detect these changes, combining electrocardiography and pulse oximetry with non-invasive beat-to-beat finger and oscillometric arterial blood pressures measurements [62]. Utilising these parameters, it creates a score on a linear scale (0–100), assessing adequacy of intra-operative anti-nociception. Initial research predominantly concentrated on its ability to guide opioid use in reducing intra-operative movement, tachycardia and hypertension [26, 27]. It is a potentially useful tool, again using standard intra-operative monitoring.

Surgical plethysmographic index

The surgical plethysmographic index (previously surgical stress index) employs photoplethysmographic waveforms of peripheral oxygen saturation measurements to analyse pulse wave amplitude and interval [28]. From these values, it generates a number on a linear scale (0–100), where values exceeding 50 are thought to represent pain. Most current research has investigated its ability to aid titration of intra-operative remifentanil this response to noxious stimulation [30, 63–68]. Although pre-clinical and clinical (intra-operative and postoperative) research indicated its ability to discriminate strong noxious stimuli from no stimulation [67–71], the method could not consistently differentiate stimulus intensities [29, 31, 72, 73]. The surgical plethysmographic index is not specific to nociception, and is influenced by both peripheral and central sympathetic tone [67, 74], sex [47, 75], intravascular volume status [76], heart rate [77], drugs [77, 78], location of the probe [79], posture [74], levels of consciousness and in awake patients, pain anticipation and emotional stress. Although the non-invasive nature of the clinical application is appealing, this is offset by large inter-patient variability.

Skin conductance

Sweating occurs as a consequence of the activation of the autonomic nervous system by noxious stimuli. This both reduces the electrical resistance of the skin and increases its conductance. Fluctuations of amplitude and frequency of skin conductance that change with stimulation can then potentially be used to assess pain [32]. Measurement involves attaching self-adhesive electrodes to the palm of the hand or sole of the foot. A filtered and processed conductance signal is then generated that responds within a few seconds, changing this frequency of fluctuations into a measured unit [32, 80, 81]. The signal is thought to be independent of adrenergic agents, haemodynamic variability and respiratory rate, as sweat glands are controlled by muscarinic receptors [32]. These, however, are not pain specific [82, 83], and can be affected by skin quality, moisture levels and environmental temperature. Outputs are also highly individual [36], and therefore interpretation should focus on each patient's own variability, rather than on isolated values or comparisons between individuals. Initial evaluation demonstrated a good ability to differentiate between the presence and absence of pain in adult postoperative patients [33, 84]. Replication of these promising results, however, has been lacking [85, 86], and there are inconsistencies in responses in paediatric postoperative patients and neonates [34, 35, 80, 83, 87–95]. Furthermore, despite clinical data that indicate a high sensitivity and specificity for noxious stimuli [96–99], depth of anaesthesia and neuromuscular reversal agents can influence results [85], and only a small correlation is demonstrated to occur with opioid dosing [97, 98, 100, 101]. Additional technical problems that cause high levels of artefact include electrode dislocation, stretching of wires and excess sweating [82, 102]. Although suitable for all ages, this tool currently represents only a crude detector of pain, with further modifications and validation required before it can be implemented clinically.

Pupillometry

Pupillary dilatation is sympathetically mediated in awake patients, and could be used to evaluate sympathetic stimulation as a consequence of pain. This rationale has led to the development of an infrared video-pupillometer; it is described as a portable, easy-to-use and non-invasive device. It measures both pupillary diameter and the light-induced pupillary dilatation reflex, shown to change in response to noxious stimuli [103–106]. However, the pupillary response can be influenced by drugs, including analgesic, antiemetic, anticholinergic and vasoactive agents, environmental luminance, age and rare entities such as Horner's syndrome [107, 108]. In addition, the mechanism of pupillary response in sedated patients remains unclear. Despite consistent results in response to transient painful stimuli [37, 38, 60, 103], pupillary light reflex amplitude has shown variable correlation with numerical rating scores in the postoperative setting [39, 40]. In the light of these results, along with issues of practicality, particularly in uncooperative patients, its clinical application remains uncertain.

Biopotentials

Biopotentials are electric potentials that transfer information between living cells. They are measured as electrocardiography, electro-encephalography (EEG) or electromyography (EMG), and can be incorporated into methods that aim to assess responses to nociception and pain (Table 2).

Nociception flexion reflex threshold

The nociceptive flexion reflex threshold provides an indication of an individual's nociception threshold, by assessing the protective withdrawal reflex [109]. Stimuli are applied via a needle or surface electrode to the sural motor nerve. This elicits a withdrawal of the biceps femoris muscle that is quantified using EMG [123, 124]. The flexion reflex threshold can be modulated by emotionally stimulating pictures or smell [111, 125], habituation stimulation [112], analgesic medications [126–130], sex [131], ethnicity [132], obesity [133], sedentary lifestyle [134], chronic pain [135, 136], cardiac cycle [137, 138], sleep [139], site of stimulation [140, 141] and circadian rhythm [142]. Furthermore, no standardised scoring method currently exists [124, 143–145]. Despite these drawbacks, scores appear to correlate well with self-reported pain, but routine clinical practice is hindered by the lack of a commercially available real-time monitoring system.

Evoked potentials

Evoked potentials assess neuronal responses as measured by EEG that occur following specific sensory stimuli [146]. These techniques are less expensive, and more clinically practical, than other methods of evaluating cerebral activity such as neuroimaging, and have demonstrated clinical applications, including monitoring depth of anaesthesia. However, to provide useful results, they require advanced signal processing to remove both background noise and non-cortical artefacts. With regard to pain assessment, EEG signal amplitudes correlate with nociceptive stimulus intensity, and are thought to reflect both peripheral and central processing of nociceptive inputs. Analgesic medication appears to alter these measured amplitudes [113, 147–149]; the specificity required to differentiate between nociceptive and non-nociceptive stimuli is, however, contentious [149–154]. Measurement of the stimulus response can be evaluated via a number of different methods. Currently, steady-state evoked potentials (measuring sustained changes after a periodic sensory stimulus) and single-trial, infrared laser-evoked potentials seem to be promising [114, 155–157], although they are not thought to reflect the neural coding of pain intensity [152]. In this respect, evaluation of gamma band oscillations, which show correlation with subjective pain intensity, provides a promising alternative [115]. At present, these techniques remain within the research setting, requiring careful experimental protocols, a sophisticated extraction process and high computational complexity. They are, therefore, beyond the realms of clinical application, but may provide possible avenues for development in the future.

Magneto-encephalography and electro-encephalography

Magneto-encephalography directly measures magnetic fields generated by intracellular dendritic activity, whereas EEG directly measures scalp voltage fluctuations due to extracellular ionic currents [158]. Both techniques detect increases in brain activity related to noxious stimulation [116, 159], that correspond with

Table 2 Biopotentials used in the assessment of pain.

Marker	Tool	Key findings	References
Spinal polysynaptic withdrawal reflex	Nociception flexion reflex (NFR)	Reliable measure of pain	Skljarevski and Ramadan (review) [109]
		Intra-operative anaesthesia	
		Attenuated by sevoflurane and propofol	Baars and Trapp [110]
		Awake healthy volunteers	
		Modulated by olfactory stimuli	Bartolo et al. [111]
		Affected by habituation	Von Dincklage et al. [112]
Neuronal signalling	Steady-state, laser-evoked potentials	*Awake healthy volunteers*	
	Laser-evoked potentials (LEP)	Subanaesthetic concentrations of propofol, sevoflurane, remifentanil and ketamine effect somatic and visceral LEPs	Untergehrer et al. [113]
	Single-trial, laser-evoked potentials	*Awake healthy volunteers*	
		Predicts intensity of pain perception	Huang et al. [114]
	Gamma band oscillations (GBO)	*Awake healthy volunteers*	
		Predicts intensity of pain perception	Zhang et al. [115]
Processed electro-encephalography (EEG) and frontal electromyography (EMG)	EEG	*Infant heel lancing*	
		Painful versus tactile stimulation in infants affects evoked EEG changes	Fabrizi et al. [116]
	Magneto-encephalography (MEG)	*Awake healthy volunteers*	
		Somatotopic changes in MEG and fMRI during visceral pain induction	Smith et al. [117]
		Activation patterns differentiate painful versus non-painful stimuli	Torquati et al. [118]
	Bispectral index (BIS)	*Intra-operative anaesthesia*	
		Responds to noxious stimuli	Coleman and Tousignant-Laflamme [119]
		Unable to predict motor response to noxious stimuli	Takamatsu et al. [120]
	Entropy difference: (Response entropy – state entropy)	*Intra-operative anaesthesia*	
		Responds to noxious stimuli	Takamatsu et al. [120]
		Predicts intra-operative nociception to guide remifentanil analgesia	Mathews et al. [121]
	Composite variability index (CVI)	*Intra-operative anaesthesia*	
		Responds to noxious stimuli	Ellerkmann et al. [122]

data obtained with functional magnetic resonance imaging (fMRI) [117, 118]. They have a temporal resolution in the order of milliseconds, which is superior to indirect neuroimaging methods such as functional near-infrared spectroscopy and MRI. However, they have limited spatial resolution (up to 1 cm) [160], detect only superficial cortical activity, and are liable to artefacts originating from overlying muscle contractions [161]. Magneto-encephalography is clinically impractical owing to large, immobile equipment, and the need to shield the signal generated from external magnetic artefacts, which interfere with the brain's weak femto-tesla signals. Conversely, EEG signals, although much easier to obtain and measure by the bedside, are more prone to signal distortions from the skull and non-neural matter.

Processed electro-encephalography

Processed EEG is used to monitor depth of hypnosis under general anaesthesia. The bispectral index (BIS) is a dimensionless number (0–100), derived from several cortical EEG parameters. Entropy processes raw cortical EEG and frontal EMG signals to produce two indices based on their frequency range, called 'response entropy' and 'state entropy'. Although studies have shown that nociceptive stimulation increased BIS, response entropy and state entropy, this was heavily dependent on both the baseline BIS levels [119] and the degree of concurrent hypnosis. It did not correlate with the quality or intensity of noxious stimuli [120], despite steady-state, end-tidal sevoflurane concentrations. Further analysis of entropy patterns revealed that nociception induced a significant difference in the

response and state entropy levels, termed 'entropy difference', which represents the electrical function of facial muscles (facial EMG) [120, 121]. Facial muscle activation is thought to represent inadequate analgesic subcortical blockade [120], and studies have used an entropy difference of less than 10 to titrate intra-operative opioids [121].

Two further EEG-based methods have been developed; the composite variability index, which combines BIS and facial EMG, and auditory evoked potentials expressed as A-line autoregressive index. Although the composite variability index detected noxious stimulation, and predicted haemodynamic or somatic responses (movement, grimacing, eye opening), it did not correlate with remifentanil plasma concentrations [122, 162]. However, the autoregressive index increased in both peak and speed after noxious stimuli, despite steady BIS levels [163].

Technology based on EEG has the potential to be a practical and useful method of assessing the nociceptive/antinociceptive balance. At present, however, none of the raw or processed parameters have been shown to predict levels of nociception accurately in awake or anaesthetised patients.

Neuroimaging and related methods

Neuroimaging is increasingly used to assess the correlation between functional and morphological status of the nervous system, and painful stimuli or conditions. Common methods include positron emission tomography (PET), MRI and near-infrared spectroscopy (Table 3). All assess neuronal function, and allow investigation of how activity in the spinal cord and brain changes depending on the quality [166, 169], intensity [147, 170–172], location [169] and duration [173] of painful stimuli. Comparisons between awake and anaesthetised volunteers have demonstrated that pain perception (nociception) is not influenced by sedation [174]. Nevertheless, some authors argue that brain activation due to noxious stimulation during functional neuroimaging is not nociception-specific, but part of the overall sensory process of detecting any salient trigger [150].

The 'pain matrix' [175] or 'neural pain signature' [176, 177] describe areas that are repeatedly activated during noxious stimulation. They comprise the primary and secondary somatosensory, anterior cingulated, insular [169, 170] and prefrontal cortices, as well as the amygdala [169, 178, 179]. The midbrain and brainstem are also thought to be involved, with mood and emotion influencing pain [180]. Furthermore, depression, distraction, anxiety [180–182], pain anticipation [183, 184] and the placebo effect [185] have been associated with activating the peri-aqueductal grey, hypothalamus, amygdala and diencephalon [177, 180].

Positron emission tomography

Positron emission tomography is one of the earliest neuroimaging techniques. It measures increases in cellular activity and enhanced glucose and oxygen consumption, by imaging gamma rays emitted from a rapidly disintegrating radioactive tracer. This indirect

Table 3 Neuroimaging used in the assessment of pain.

Marker	Tool	Key findings	References
Brain cellular activity	Position emission tomography (PET)	Brain network activated in acute pain	Apkarain et al. (review) [164]
		Awake healthy volunteers	
		Correlates with opioid system activation and affective pain scores	Casey et al. [165]
	Functional blood oxygenation level-dependent magnetic resonance imaging (BOLD fMRI)	*Awake healthy volunteers*	
		Neurological signature to thermal pain, modulated by opioids	Wager et al. [166]
	Functional arterial spin labelling MRI	*Awake healthy volunteers and patients with chronic low back pain*	
		Correlated with clinical pain	Loggia et al. [167]
	Functional near-infrared spectroscopy	*Awake and intra-operative anaesthesia*	
		Responds to noxious stimuli	Gelinas et al. [168]

assessment of regional neuronal activity results in a three-dimensional image of a functional process.

Extensive research using PET shows similar sensitivity in pain-related regional changes as fMRI [164, 180]. Furthermore, it has been used to investigate various pharmacological effects on the brain, such as the opioid-induced reduction in insular responses to noxious stimuli [165, 184]. Despite its clear uses in pain research, its popularity is diminishing owing to concerns regarding ionising radiation exposure and invasive intravascular injections of radioactive isotopes.

Magnetic resonance imaging

Magnetic resonance imaging relies on the principle that protons align in a strong magnetic field, and fall back into their original position when it is turned off, emitting a magnetic flux that is transduced and measured as an electrical signal. While static MRI can provide structural information about grey and white matter [186], fMRI allows quantification of neuronal activity in specific areas of the brain and spinal cord, by associating metabolic changes during cell activity with localised haemodynamic responses. It has the advantage of not requiring invasive contrast or exposure to ionising radiation.

Two main types of fMRI have been explored as potential methods to measure pain. Blood oxygenation level-dependent (BOLD) fMRI utilises the differences in magnetic properties of oxy- and deoxyhaemoglobin to determine spatial and temporal changes in cerebral blood flow [187]. Evidence suggests that these imaging signals correlate with the presence of painful stimuli, as well as their location, time frame, quality (heat versus pinprick) [169], intensity [166, 170, 179] and chronicity [173]. They also show how emotional and pharmacological stimuli modulate neuronal pain signalling [166]. This technique has good spatial coverage, allows large areas of brain and spinal cord to be seen in a single study, and can detect small differences in discrete areas over time [188]. The second method is arterial spin labelling or blood oxygenation-sensitive, steady-state techniques. Arterial water is 'magnetised' by pulsed or continuous radiofrequency radiation, and tagged proximally to the imaged segment [189, 190]. The area becomes perfused with the newly traced blood, and the labelled image is subtracted from the unlabelled form, in one imaging sequence. In pain research, arterial spin labelling has shown similar spatial–temporal distributions in brain activity to PET and BOLD imaging [191]. Its main advantages over other neuroimaging techniques are a stable and quantifiable signal, with low drift over time and between subjects, making it ideal for investigating cerebral activity in longitudinal studies that look at prolonged treatment outcomes or spatial–temporal activity patterns [192, 193]. As arterial spin labelling measures the amount of tracer within brain capillaries, the images more accurately reflect the localisation of synaptic and neuronal activity compared with BOLD MRI, which images the macrovasculature [167, 192]. However, low-amplitude signals, poor temporal resolution and a lower signal-to-noise ratio can result in less sensitivity and spatial resolution compared with BOLD techniques [192, 194]. Recently, technical refinements such as turbo-arterial spin labelling, with shorter imaging times and higher magnetic fields and filters, have improved signal-to-noise ratios and provide faster image acquisition.

All MRI-based methods are hampered by the need for bulky, expensive equipment, long investigation times and the risk of artefacts, affecting image specificity and sensitivity [192]. They have limited use in day-to-day clinical assessment of pain.

Functional near-infrared spectroscopy

Near-infrared spectroscopy is a non-invasive, indirect method of measuring localised neuronal activity. As with fMRI, it utilises changes in local blood oxygenation and haemodynamics, which are thought to correlate with regional brain activity [195]. Two chromophores, with different infrared absorption spectra, penetrate the brain via an 'optical window' and are absorbed to a variable degree, depending on the concentration of oxygenated and deoxygenated haemoglobin. Specialised light detectors convert the remaining signal into a graphic and numerical display of neuronal activity. Increases in neuronal activity raise cerebral oxygen consumption, and cause a corresponding increase in cerebral blood flow and volume, which alters the concentrations of oxygenated versus deoxygenated haemoglobin. This complex interaction, known as 'neurovascular coupling', is the fundamental

principle behind functional near-infrared spectroscopy's ability to detect local changes in brain neuronal activity [196]. Functional near-infrared spectroscopy is widely used in the assessment of brain activity in neonates and children [197]; however, its application in pain evaluation is not established. It is also currently used to study the temporal and spatial localisation of cortical activity in response to other stimuli, such as vision [198], sound [199], language [200, 201] and taste [202].

Its main advantages are a lack of exposure to ionising radiation, which allows for repeated use over extended periods of time. Functional near-infrared spectroscopy shows promise as a tool for independently assessing pain in adults [168] and children [203], when self-reporting is not possible [204]. Its employment as a continuous, bedside monitor would be of particular use in critical care or intra-operative settings.

Biomarkers

A biomarker is broadly defined as "*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention*" [205]. The range of biological parameters covered by this definition spans from genotyping to implementing and scoring a clinical scale, making most biological variables potential biomarkers. Within pain medicine, biomarkers could encompass self-reported pain intensity scores, changes in physiological variables and functional brain imaging. However, biomarker research has the potential to develop truly objective pain measures, by using an integrated systems approach that focuses on the 'onomics': measuring genetic or protein responses, or metabolic products, at cellular level (Table 4). Systems biology aims to quantify molecular elements of a biological system, and integrates these to serve as predictors to explain emergent behaviours [210]. Nociception is complex, involving varying transduction mechanisms and mediators, depending upon the cause, nature and location of the stimulus. A systems biology approach is therefore well-suited to develop markers that could identify the presence and intensity of pain, specific to each potential nociceptive mechanism. Creating an easy-to-sample, quick to measure, sensitive and specific marker could represent the 'holy grail' of pain assessment. However, this approach is not without problems, including potential inter- and intra-individual variation in marker response, and also methodological issues, such as determining the markers' specificity for pain.

A number of avenues could be exploited to develop pain biomarkers. The most obvious is the stress

Table 4 Biological ('bio') markers used in the assessment of pain.

Marker	Tool	Key findings	References
Stress hormonal and metabolic changes	Assays	*Venepuncture in children* Salivary alpha-amylase levels elevated in conjunction with elevated pain scores	Ferrara et al. [206]
		Awake healthy volunteers Insulin sensitivity decreased, and serum cortisol, free fatty acid, plasma adrenaline, glucagon and growth hormone increased, following noxious stimulation	Greisen et al. [9]
Drug effect-site concentrations	Noxious stimulation response index (NSRI)	*Intra-operative anaesthesia* Higher probability of predicting a response to tetanic stimulation than BIS or acoustic evoked potential index	Luginbühl et al. [207]
Biochemical analytes	Serum lipid levels	*Hospitalised patients* Two groups (acute visceral pain and somatic pain) both showed increased serum lipid levels during periods of persisting pain	Krikava et al. [208]
Inflammatory mediators	Immunoassays	*Awake healthy volunteers* Differing release patterns of interstitial cytokines following inflammatory and noxious heat stimulation	Angst et al. [209]

response, which involves two main systems; the hypothalamic-pituitary axis and the sympathetic nervous system. A relationship exists between stress and pain, whereby the release of stress hormones could reflect the presence of pain. Serum catecholamine levels reflect sympathetic activation, and could be measured in an attempt to evaluate pain. However, a less invasive surrogate marker of this response is salivary alpha-amylase [211], which has shown promising results in the paediatric setting [206]. In addition, in an experimental setting, acute pain without tissue injury has been linked to increased release of cortisol, adrenaline and free fatty acid, with decreased insulin sensitivity [9]. However, the strength of the link between sympathetic activation and pain has recently been questioned in acute pain research [212]. Pain may only represent a small part in this complex neuroendocrine response, and therefore using these hormonal changes as specific biomarkers of pain may be futile.

As biomarker research is heavily embedded in pharmacological discovery, measuring drug concentrations, especially at effector sites, poses another area for development of pain biomarkers. While establishing serum drug concentrations reflects pharmacokinetic and pharmacodynamic processes, it remains unclear how this would reflect pain in the clinical setting. A novel approach is the noxious stimulation response index. This univariate index is calculated from weighted propofol and remifentanil concentrations, and aims to show the synergism of using a hypnotic and opioid to suppress an individual's response to noxious stimuli [207]. However, as this requires administration of hypnotic agents, it reflects anaesthesia as much as nociception, and is not necessarily useful for evaluating pain specifically in a clinical setting.

Potentially, any biochemical analyte could be used as a biomarker, and to this end, many agents routinely measured have been explored with respect to pain. Most notably, changes in serum lipid levels may increase in parallel with increasing pain intensity. However, preliminary work has been hampered by numerous confounding factors, including co-morbidities, that could be driving these lipid changes, rather than pain [208].

Finally, there is a well-established link between inflammation and pain. Historically, pain has been used as a clinical sign of inflammation, and evidence supports the hypothesis that pro-inflammatory cytokines both induce and facilitate pain and hyperalgesia, through direct and indirect action on peripheral nociceptors [213]. Cytokines and chemokines are released peripherally in experimental inflammatory pain, and could serve as potential biomarkers [209]. Results from the clinical setting, however, are more complex. Acute and chronic conditions associated with pain and inflammation demonstrate variable release of pro- and anti-inflammatory cytokines, not always consistent with measured pain intensity [214, 215]. In addition, evidence is hampered by methodological inter-study inconsistencies, including samples ranging from direct serum level analysis to skin biopsies, and the use of heterogeneous populations with conditions that are difficult to define, such as fibromyalgia. This field remains promising for further development, however, once these sampling and investigational inconsistencies are resolved. Other areas of promise include evaluating the inflammatory prostaglandin metabolic pathway, the products of which may reflect pain [216]. Overall, however, while there is theoretical promise in exploring biomarkers as an objective measure of pain, it is unlikely that there will be one specific mediator that reflects pain alone, and it may be more useful to focus on mediator profiles in a more systems-based approach.

Comparison of different assessment tools

New pain assessment tools are validated in conscious patients by comparing self-reported pain scores with the output of the novel device. This is not possible in sedated or cognitively impaired patients, as no gold standard exists in this patient cohort. To address this problem, comparison between tools has been used, but as this lacks a validated method, the results should be interpreted with caution (Table 5). The majority of published studies compare the surgical plethysmographic index with either haemodynamic responses, EEG derivatives or concentrations of opioids [67, 219, 220]. The surgical plethysmographic index has demonstrated greater sensitivity in detecting nociceptive stimuli and better correlation with effector site remifentanil and alfentanil concentration during general anaesthesia than heart rate and entropy values [68, 69]. Some initial results even suggest that it can detect responses to phar-

Table 5 Comparison of different tools used in the assessment of pain.

Tools compared	Key findings	References
NFRT and BIS	*Intra-operative anaesthesia*	
	Comparable prediction of movements in response to noxious stimuli	Von Dincklage et al. [217]
NFSC and SPI	*Postoperative*	
	Both only moderate sensitive and specific to pain	Ledowski et al. [73]
NFSC, SPI and plasma stress hormones	*Intra-operative anaesthesia*	
	Response of NFSC but not SPI to fentanyl bolus. Both only minimally associated with plasma stress hormone levels	Ledowski et al. [100]
NFRT, BIS, CVI and NSRI	*Intra-operative anaesthesia*	
	NFRT as best predictor of movement and HR responses to noxious stimuli	Von Dincklage et al. [218]
PRD and ANI	*Intra-operative anaesthesia*	
	Correlation of both with regional anaesthesia failure (children)	Migeon et al. [60]
NFSC and ANI	*Intra-operative anaesthesia*	
	ANI more sensitive for intra-operative stimulation in children	Sabourdin et al. [61]

NFRT, nociception flexion reflex threshold; BIS, Bispectral index; NFSC, number of fluctuations of skin conductance; SPI, surgical plethysmographic index; CVI, composite variability index; NSRI, noxious stimulation response index; PRD, pupillary reflex dilatation; ANI, analgesic nociception index.

macological interventions such as fentanyl boluses, although this has recently been questioned [78, 100, 221]. In postoperative patients, both the surgical plethysmographic index and fluctuations of skin conductance have been shown to differentiate grossly between the extremes of pain [31, 33, 84], but not between more subtle differences. Furthermore, fluctuations of skin conductance, but not the surgical plethysmographic index, were correlated with self-reported pain intensity [73].

When looking at other potential tools, the nociceptive flexion reflex threshold appears a better predictor of movement and heart rate responses to noxious stimuli under general anaesthesia than either the noxious stimulation response index or propofol–remifentanil effect-site concentrations [218]. However, it has been criticised as being more specific for muscular activity, rather than detecting pain due to measuring a muscular reflex.

Stress hormones such as cortisol, adrenocorticotropic hormone, adrenaline and noradrenaline have been used to evaluate the magnitude of surgical stress [222]. However, as stress hormone levels are influenced by a number of factors other than pain, there is conflicting data regarding their correlation with both surgical plethysmographic index and skin conductance [96, 100, 212, 219].

Currently, most tools are difficult to evaluate in specific clinical situations, and comparing these tools with each other does not necessarily address the issue of validation.

Composite algorithms

As individual physiological variables are unlikely to become validated markers of nociception alone, algorithms that encompass several parameters might provide an alternative solution (Table 6). Furthermore, it has been suggested that combining multiple physiological parameters better reflects the complex nature of pain. Statistical modelling and data analysis have, for instance, been used to create the response index of nociception, which utilises heart rate variability, skin conduction and EEG, and also the nociception level index, which evaluates heart rate variability, skin conduction and photoplethysmography. These multi-variable approaches appear to be superior predictors of pain intensity and intra-operative nociception to any individual parameter alone [220, 221, 223–225], but evidence so far comes only from uniform patient populations who have undergone a limited array of surgical procedures or noxious stimuli, under a single type of anaesthesia. Although this approach reduces inter-patient variability experimentally, it supports the notion that even these tools are influenced by a plethora of confounding factors. Therefore, the clinical value of composite tools remains to be determined, especially in more heterogeneous patient populations.

Table 6 Composite algorithms used in the assessment of pain.

Tools combined	Key findings	References
ECG, PPG, EEG (RE)	*Intra-operative anaesthesia*	
	Better correlation with noxious stimuli than single variables	Seitsonen et al. [221]
HRV, SE, RE, PPG (RN)	*Intra-operative anaesthesia*	
	Correlation with noxious stimuli and effect-site concentrations of remifentanil	Rantanen et al. [223]
HRV, SE, RE, PPG (RN)	*Intra-operative anaesthesia*	
	Correlation with intra-operative noxious stimuli and predicted patient movement	Saren-Koivuniemi et al. [224]
Linear combination of HR, HRV, NFSC, PPG	*Awake healthy volunteers*	
	Significant differentiation between mild, moderate and severe pain (tonic heat stimuli)	Treister et al. [220]
HR, HRV, NFSC, PPG (NoL)	*Intra-operative anaesthesia*	
	Better correlation with moderate to severe noxious stimuli than single variables	Ben-Israel et al. [225]

EEG, electro-encephalography; PPG, photoplethysmography; RE, response entropy; HRV, heart rate variability; SE, state entropy; RN, response index nociception; HR, heart rate; NFSC, number of fluctuations of skin conductance; NoL, nociception level.

Discussion

Studies have repeatedly shown that at any time, 25–40% of patients admitted to hospital suffer moderate to severe pain [226]. Management is often hampered by poor assessment, especially in patients who are unable to self-report [227]. As a consequence, clinicians and scientists alike have identified the need to develop more objective measures of pain, to aid its management. As a consequence, a wide variety of tools employing physiological parameters linked to pain are currently undergoing investigation, of which this article gives an overview of important trends.

A valid test requires high and reproducible sensitivity and specificity, with a strong probability that the parameter will correlate with pain intensity [228]. However, investigating objective nociceptive measures poses a multitude of challenges.

First, as pain is a conscious experience involving considerable psycho-social components [1], sedated or unconscious patients by definition cannot experience it. Under these circumstances, it is more accurate to talk about nociception, the process that transmits a noxious stimulus to higher brain centres, where it is modulated. Some would suggest that managing nociception is not important, as it is the conscious process of pain that is distressing for the patient. However, a body of research suggests that not managing nociception can lead to central changes in pain pathways that predispose individuals to chronic pain states [229, 230]. In anaesthetised and sedated patients, the physiological changes that occur are therefore a consequence of nociception rather than pain. This distinction is important, as it implies that using self-reported pain assessments to validate tools that are most likely to assess nociception is incorrect. Yet, this is the gold standard applied to evaluating all such 'objective' tools. Not surprisingly, therefore, some tools such as pupillometry and skin conductance show inconsistent correlations with pain intensity ratings in awake patients. While the lack of a gold-standard comparator already hampers the development of new assessment tools in anaesthetised patients, the situation becomes even more complex in the confused or non-verbal patient. Here, the question arises as to what degree self-reporting is accurate, and can hence be used for method validation. Usefully, even moderately confused patients have been shown to be capable of using rating scales [3], and thus careful patient selection might be the key to develop and validate new tools.

Currently, potential tools and algorithms employ variables that are by nature only indirect measures of pain or nociception, and hence are not necessarily specific. This leaves them vulnerable to the influence of other factors, such as medication or disease processes [5]. It is often difficult to determine which of the observed changes in the parameter under investigation are genuinely due to pain, and which are a result of pathological, pharmacological or physiological events.

In addition, many analgesics are also sedatives [231], which complicates matters in the intra-operative and critical care settings, where tools are needed that can reliably separate analgesia from sedation. Validation is further complicated by the fact that new techniques frequently rely on the same surrogate variables of pain as the old methods they aim to replace. As a consequence, these new tools may be hindered by the same confounding factors that made their predecessors inaccurate.

Many available tools use mathematical algorithms that either average observations over time or employ thresholds or cut-offs to derive their values. This imposes the risk of missing transient or small physiological responses that could indicate nociceptive stimulation. Some techniques are held back by technical issues. For instance, the bulkiness of the equipment prevents MRI from being adopted into everyday pain assessment. Finally, the anatomical, physiological and functional connections of the nociceptive and autonomic nervous systems warrant some special considerations. This notion is based on findings that a considerable number of alternative assessment tools employ variables that are directly related to autonomic function. These work on the premise that a painful stimulus will elicit certain autonomic responses that can be measured and integrated as physiological markers of nociception. This long-held, widespread belief largely rests on evidence produced 30–40 years ago in intra- and postoperative settings. This association is debatable, as there are few published data looking at autonomic stimulation following pain without tissue injury. Although the rationale behind the functional connection of the two systems is plausible [232], it can also be suggested that changes in autonomic activation are the result of tissue damage, rather than of pain itself. Nevertheless, evidence to date suggests that autonomic responses to nociception are binary in nature, detecting its presence but not its severity [232]. However, a tool that does not correlate with the stimulus intensity is of limited value in clinical practice. Here, combinations of different parameters may be helpful, but more research is needed to address this issue.

Although there are some promising results available with most new methods, there are limited data to suggest that they can improve clinical care. Although some can objectively indicate the presence of pain or nociception, this should not be the main goal of developing these tools. If we as clinicians are to improve our ability to manage pain in difficult settings, we need to have methods that do not just demonstrate pain as a black or white phenomenon, but in various shades of grey.

Conclusion

Although clinically needed and theoretically promising, currently there is not enough evidence to support the widespread use of any physiological markers as 'objective' measures of pain and nociception. This is despite recently increased efforts, raising the question whether this is possible in the foreseeable future. Nevertheless, there are some promising avenues on the horizon. Biomarker research as part of clinical phenotyping, and the development of composite algorithms, should be closely watched.

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