Harnessing the power of biomolecules for acute pain monitoring

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Abstract

Background and Aims: Pain is a huge clinical issue around the world. An accurate, reliable and valid pain assessment is vital when it comes to accurate diagnosis and sufficient treatment outcomes. The self-report pain assessment is still the gold-standard to assess pain intensities. However, this method could not be adopted in patients such as infants, nonverbal elder patients with cognitive impairment and nonconscious patients in ICU, thus a new pain assessment method is requited to assess pain intensities in those patients.

Monitoring the concentration of biomolecules such as cortisol, substance P, ATP could be an ideal choice as those biomolecules play vital roles in nociceptive pain pathways. Besides, the concentration of these molecules are easy to monitor via plasma and saliva, thus is appropriate for clinical and research use. This article aims to summarize the relevant studies to explore whether there is a potential to use these biomolecules for subjective assessment of acute pain.

Methods: Pubmed, Web of Science and Medline were searched to obtain articles relevant to the topic area.

Results: 892 articles have been identified by the databases. Then 749 articles were identified after removing duplicates with Endnote X8. After screening for the abstracts, 18 articles were chosen for full-text reviewing. Then 11 articles have been reviewed.

There are three studies relevant to plasma cortisol and acute pain intensities, and all three studies report a positive relationship between plasma cortisol level and acute pain intensities.

There are three studies related to plasma substance P (SP) and acute pain intensities, among which two studies report that the plasma SP is positively correlated to acute pain levels. Only one study is relevant to saliva SP but no significant relationship is observed between saliva SP and acute pain intensities.

One study is identified related to serum ATP and acute pain intensities. And an increased level of serum ATP is observed in patients with higher acute pain intensities.

Summary and Conclusions: For cortisol, there is sufficient evidence to support the potential of using plasma and saliva cortisol as a biomarker to monitor acute pain intensities. The plasma and saliva cortisol baseline levels can be affected by various factors such as age, gender, circadian rhythm and sex-hormones. Thus cortisol might not be suitable to assess acute pain intensities in infants due to the unstable cortisol releasing pattern in infants. But the cortisol may be appropriate to assess pain intensities in both older patients with cognitive impairments and unconscious patients in ICU as the age-related and gender-related changes in cortisol baseline levels have been widely studied.

For SP, evidence shows the potential of using plasma SP to assess acute pain intensities while more studies are required to study the impact of aging and gender difference on the baseline level of plasma SP level. However, there is a lack of evidence to support the potential to use saliva SP as a biomarker to monitor acute pain intensities. More studies are required in this area and future studies are recommended to take the different saliva collecting methods into account, which might lead to different SP concentration.

For ATP, it's hard to summarize the possibility to use ATP as a biomarker of acute pain intensities due to the lack of evidence. Future studies are recommended to concentrate on the relationship between plasma and saliva concentration of ATP and acute pain related to inflammation and tissue injury

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Abbreviations :

American Geriatric Society - AGS

Adrenocorticotropic hormone - ACTH

Central nerve system - CNS

Corticotrophin-releasing hormone - CRH

Corticosteroid-binding globulin - CBG

Chronic wide spread pain - CWP

Excitatory amino acid transporters - EAAS

Hypothalamic–pituitary–adrenocortical axis – HPA axis

Ionotropic receptors - iGluRs

Metabotropic receptors - mGluRs

Numeric rating scale - NRS

Osteoarthritis - OA

Substance P - SP

Sickle cell disease - SCD

Verbal categorical rating scale - VRS

Visual analogue scale - VAS

N-methyl-D-aspartate receptors – NMDA receptors

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors – AMPA receptors

Introduction:

Pain, referring to “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Bogduk et al., 1994), has become a significant social, economic and clinical issue worldwide (Henschke et al., 2015). Typically, pain can be classified into acute pain and chronic pain. Chronic pain refers to the pain continues after normal healing time, and lasts or reoccurs for over three months after noxious stimuli (Bogduk et al., 1994). Acute pain refers to the pain occurs after noxious stimulus and related to surgery, trauma or acute illness, which is self-limited (Grichnik and Ferrante, 1991)

For both acute pain and chronic pain, an accurate validated and reliable pain assessment is greatly beneficial as it can help clinicians to:1) better understanding the pain mechanisms; 2) accurately diagnose and treat patients; 3) choose the most sufficient and personalized therapy based on individual variety and 4) monitor the outcome of a therapy subjectively and accurately. Generally, pain can be assessed via various factors including location, duration, and intensity, among which pain intensity is the most commonly assessed factors with current assessment tools. And current methods to evaluate pain intensities in both adults and children will be discussed in the following paragraph.

1. Pain assessment in adults

Currently, there are three tools used to measure adult pain intensity. These include: visual analogue scale (VAS), numeric rating scale (NRS) and verbal categorical rating scale (VRS) (Breivik et al., 2008).

VAS pain rating sale consists of numbers from 0-10 and 0-100 with zero representing " no pain" and the end point representing the "worst possible pain". As shown in Figure 1, the VAS consists of a 10 cm vertical or horizontal line, on which patient can draw a mark to represent their pain levels. The length of this mark in centimeters will be used to assess their pain intensities (Jensen and Karoly, 2011). Compared to other pain rating scale, the ratio

scale properties of VAS is a major advantage, making it more accurate and reliable when comparing scores at different times or from independent subjects. However, it's important to note that VAS is not suitable to monitor pain in patients with perceptual- motor impairments or in patients with cognitive deficits (Katz and Melzack, 1999).

VRS rating scale includes a list of adjectives to describe pain intensities such as none, mild-moderate and severe. A patient could choose from the list to represent their pain levels. Then the adjectives are assigned numbers. One disadvantage of VRS is that the numbers could result in a misunderstanding that the intervals between the descriptors are equal, which could be a cause of error (Jensen and Karoly, 1992).

NRS is a scale consists of 11,21 or 101 numbers with end points representing no pain or worst pain (Williamson and Hoggart, 2005). Patients are typically asked to choose the numbers to represent their pain intensities. Compared to VAS, NRS is easy to conduct but is not able to present the subtle changes in pain intensities (Breivik et al., 2008).

Currently, VAS, NRS and VRS have all been proven to be reliable and valid in the clinical assessment of pain intensity. However, the common shortcoming is that these methods are not suitable for patients in comma, or those with cognitive impairments such as dementia and delirium.

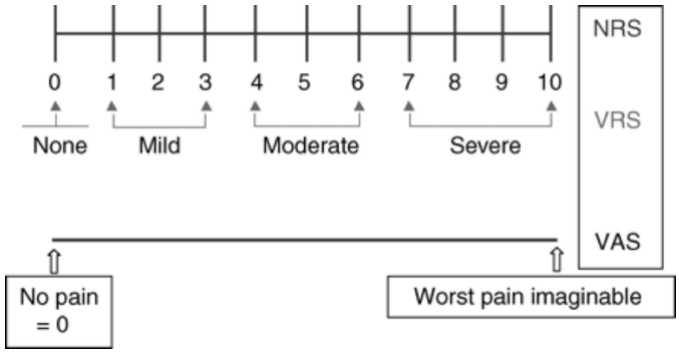

Figure 1(Breivik et al., 2008): NRS, VRS and VAS pain rating scales

1.1 Pain assessment in older patients with cognitive impairment

In cognitively impaired patients, self-report pain assessment is impossible, thus observations are required to determine pain intensity. American Geriatric Society (AGS) recommends the assessment of 6 behaviors in order patients with cognitive impairments including "facial expressions, verbalizations and vocalizations, body movements, changes in interpersonal interactions, changes in activity patterns and routines, and changes in mental status" (Lichtner et al., 2014). Based on these behavioral domains, various assessment tools (Appendix 1) have also been designed to assess pain in patients (for review see (Herr, 2011)). Recent reviews, however, analyze those tools and report that although some of the tools are with promise such as PAINAD scale, none of the tools could be recommended over others in clinical practice due to the lack of reliability and validity (Somes and Donatelli, 2012, While and Jocelyn, 2009).

1.2 Pain assessment in unconscious patients in Intensive Care Units (ICU)

Similar to the older patients with cognitive impairments, self-report pain assessments in patients in ICU with brain injury or critical illness is not possible either. Generally, when the self-report of pain intensities is impossible, patients in ICU will be assessed based on several pain indicators (Appendix 2) including facial expression, body movement, muscle tension and vocalization (Roulin and Ramelet, 2012). Based on the indicators, eight assessment tools have been designed to assess pain in unconscious patients ICU, among which Behavioral Pain Scale (BPS) and the Critical-Care Pain Observation Tool (CPOT) have gained great popularity (Gélinas et al., 2013). The pain indicators and behavioral assessment tools have been approved to be valid and reliable to detect pain in unconscious patients with critical illness, although more evidence is required in the future (Gelinas, 2016). However, these behavioral tools and indicators have been demonstrated to be insufficient to represent pain levels in unconscious patients with brain injuries, dementia and delirium (Roulin and Ramelet, 2012).

2. Pain assessment in children

The acute pain assessment in children can be classified into three categories: self-report measurement, behavioral assessment and biological assessment of pain.

2.1 Self-report assessment

Self-reporting pain assessment in children consists of various methods such as VAS, NRS, category rating scales including FACES scale and multiple-sized, and non-verbal self-reporting pain assessment via drawing (Appendix 3). The major advantage of these tools is that they are easy to conduct and use in clinical practice. However, they are limited by the age and developmental level of children, and cannot be used on children under three years old or those with a cognitive impairment. Additionally, bias could happen in the self-reporting measurement as children could deny pain out of fear of further treatment (McMahon, 2013). Moreover, due to the lack of clinical evidence, it has yet to be proven whether one method is more recommended than another (Brand and Al-Rais, 2019).

2.2 Behavioral assessment in children

Behavioral assessment of pain intensities is suitable for children younger than six years old or those with serious cognitive impairments (McMahon, 2013). To date, various observation tools have been invented, among which FLACC sale (Appendix 4) has been demonstrated to be reliable and valid and has since, become widely used in clinic practice (Brand and Al-Rais, 2019).

Specific assessment tools (Appendix 5) have also been designed to assess pain in children which cognitive impairment including r-FLACC, INRS, NCCPC-PV, NCCPC-R and PPP and Pediatric Pain Profile (Hauer and Houtrow, 2017). However, recent review summarizes that none of the tools is demonstrated to be superior to another, although r-FLASS and NAPI

have been rated with higher clinical utility by physicians and nurse(Hauer and Houtrow, 2017). Moreover, for the infants, pain is assessed via their facial expressions and gross body movements, but these behaviors can be easily affected by factors other than pain such as fear, hunger and thirst (McMahon, 2013).

2.3 Biological assessment in children

As shown in Table 1, biological assessment measures pain using various factors such as heart rate, transcutaneous oxygen, sweating and stress response (Wall et al., 2006). However, biological assessment has the same problem as behavioral assessment: hard to determine the cause of the change in the biological factors. Moreover, they are not frequently used in the clinical setting thus is not validated as other methods.

Indicator	**Descriptor of pain**
Heart rate	Increase in heat rate
Transcutaneous oxygen	Reduced in transcutaneous Oxygen
Sweating	Palmar sweating
Stress response	Unstable plasma cortisol concentration

Table 1：Biological assessment in children and infants

To summarize, self-reporting pain assessment is still the gold-standard to assess pain in patients. However, in both older and younger patients with cognitive deficits, self-reporting assessments cannot be used and thus, additional tools are required to assess one's overall pain intensity. Interestingly, behavioral and biological assessment tools have since been used in such patient to overcome this issues. However, a lack of clinical support has questioned the sufficiency and reliability of these methods, thereby outlining the need for further research. It has also been shown that behavioral and biological assessments can be

easily influenced by factors like stress, hunger and thirst, and are likely to lead to high levels of bias or error. Last but not least, biological assessment is hard to conduct in normal clinical settings, thus might not be suitable for daily use.

Therefore, a new method to detect pain in those vulnerable group should be considered. The method should be reliable, validated and easy to perform in clinical practice. Monitoring the concentration of biomarkers such as cortisol and neural-transmitters is an ideal choice for qualitative and objective assessment of pain, because the biomolecules can provide real-time information about changes in the neural system. Additionally, various studies have been carried out to explore the potential to monitor pain intensities via the concentration of several biomolecules in plasma and saliva, which makes the method more practical and flexible to use in the clinical and research setting. As the current studies related to this area mainly focus on the biomolecules including cortisol, substance P and ATP, I will focus on these biomolecules. In the following chapter, I will give a brief introduction of the roles of cortisol, substance P and ATP in acute pain.

3. Potential Biomarkers to monitor acute pain

3.1 Cortisol

Cortisol, an essential hormone taking part in various biological reactions including metabolic activities, anti-inflammation activities and stress response upon noxious stimuli, is one of the most significant physiological products produced by the middle zone of the adrenal gland (Perry and Medbak, 2013). The secretion of cortisol is controlled by hypothalamic–pituitary–adrenocortical (HPA) axis (Stephens and Wand, 2012). As is shown in Figure 2, upon stimulation, the hypothalamus will release corticotrophin-releasing hormone (CRH), which activates pituitary to synthesis and release adrenocorticotropic hormone (ACTH) (Miller et al., 2016). Here, the adrenal cortex is then targeted by ACTH to release cortisol, which in

turns, provides a negative feedback to suppress the synthesis and release of CRH and ACTH from hypothalamus and pituitary gland, respectively (Miller et al., 2016).

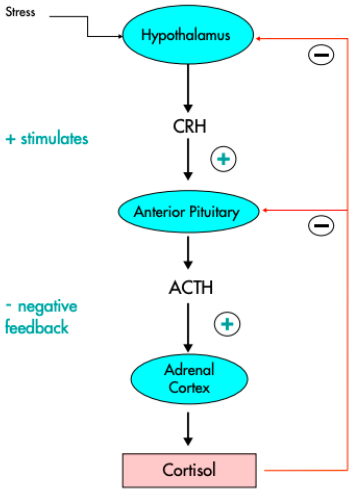

Figure 2 (Perry and Medbak, 2013): Interactions within the HPA axis

With a stimulation of a pain-related or non-pain-related stress, the sympathetic nerve system will be activated initially to evoke a fight or flight response, then the HPA axis will be activated subsequently and the cortisol will be released from adrenal cortex, providing sufficient energy to cope with the stress and escape from the potential dangers (Blackburn-Munro and Blackburn-Munro, 2003, Jankord and Herman, 2008). Additionally**,** with stimuli such as acute pain, due to the negative feedback to the HPA axis, the cortisol level will return to baseline rapidly (McEwen, 1998). In contrast, with chronic stress such as chronic pain, exaggerated negative psychological factors could sensitize the brain to stressors and prolongs the HPA axis activation (Anisman and Merali, 2002). As a result, there will be a rapid exhaust of cortisol, followed by deficient feedback to HPA-axis. This eventually leads to decreased cortisol concentration, along with cortisol dysfunction characterized by increased unbound (insufficient) cortisol, reduced negative feedbacks, and increased inactive cortisol receptors (McEwen and Kalia, 2010, Yang et al., 2012).

3.2 Substance P

Substance P (SP) is an 11 amino acid which belongs to Tachykinin family, a large family of several closely related peptides and characterized by the sequence of -Phe-XXX-Gly-Leu-Met (Severini et al., 2002). There are three receptors that mediate the biological activities of tachykinin families: NK1, NK2 and NK3. SP has a prevalence for NK1 receptors, but it can activate all three receptors under certain conditions(Regoli et al., 1994). Generally, SP is synthesized in neurons of dorsal root ganglion, after which it is stored in vesicles and transported to peripheral and spinal nerves endings (Hoyer and Bartfai 2012). SP is involved in various biological procedures such as vasodilation, smooth muscle contraction and immune response (Payan, 1989, Li et al., 2014, Jovas et al., 1995, Marriott and Bost, 2001)

Additionally, SP is also an essential neurotransmitter in pain signal transmission. Released by A or C fibers at peripheral endings or spinal dorsal horn, SP acts as an excitatory neurotransmitter for nociceptive input and the response of SP towards stimuli is rapid and short-lasting (Okeson, 2014). As well as this, SP is a vital part of neurogenic inflammation. Upon an inflammation and tissue injury, SP is released at peripheral endings and stimulates the release of serotonin and histamine; factors which can influence the adjacent nociceptive neurons, leading to a broader painful area (Okeson, 2014). Furthermore, in inflammation, NK1 receptors will increase, which could couple to phospholipase C. This will which in turns activate N-methyl-D-aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, contributing to neural plasticity (Weisshaar and Winkelstein, 2014). Recent research also finds that via inducing a slow development of NMDA receptors, SP is closely associated with neural plasticity and thus, is closely related to chronic pain(Dasgupta et al., 2017).

3.3 ATP

ATP, as a neurotransmitter, participates in both acute pain and chronic pain, which is conducted via various receptors (Figure 3). Among these, as shown in Figure 3, is P2X3, a major receptor that is relevant to acute pain. It is widely located in the peripheral nerves, spinal cord and trigeminal brainstem (for review see(Burnstock, 2016).

Upon acute stimuli, ATP is released by afferent fibers, damaged neural or non-neural cells, after which it binds to the P2X3 receptors and transfer the pain signals to the dorsal horn. Then at dorsal horn, ATP continually combines with the receptors on the secondary fibers, furtherly transferring acute pain signals (Wirkner et al., 2007).

P2X3/P2X2/3	Terminals of nociceptive sensory nerves	Mediation of acute and chronic visceral, musculoskeletal, and dermal pain initiated by purinergic mechanosensory transduction and cancer
	Sensory nerves in CNS	Mediation of neuropathic and inflammatory pain, including migraine
P2X4	Microglia in CNS	Mediation of neuropathic pain
P2X7	Microglia	Mediation of neuropathic pain
	Tumor cells	Mediation of cancer cell death
$P2Y_{12}$	Microglia	Mediation of neuropathic pain
P1		
A_1 agonists	Presynaptic terminals of peripheral and central neurons	Inhibitory modulation of pain pathways
A_2 antagonists		Antinociception

Figure 3 (Burnstock, 2016): Receptors of ATP

In previous research, increased peripheral injection of P2X receptors agonists have been shown to increase pain sensation and pain responses in rat models (Bland-Ward and Humphrey, 1997). Additionally, ATP receptors are only demonstrated to be associated with acute pain related to tissue injury and inflammation but not response to acute mechanical

stimuli. This is due to P2X3-null rat showing no difference in the behavioral responses towards acute stimuli(Souslova et al., 2000).

Based on above, cortisol, substance P and ATP have been shown to vital roles in pain signal transmission and thus, have the ability to monitor pain levels. With no limitations in regard to patient selection, it can be suggested that these biomolecules are therefore appropriate for those not suitable for traditional pain assessment methods. Lastly, as these molecules are present in plasma and saliva, they are easy to collect within clinical practice and thus, can be analyzed accordingly.

Despite being advantageous, no reviews have been conducted to summarize recent breakthroughs and difference between these biomarkers. It is for this reason, this review aims to: 1) explore whether the plasma and saliva concentration of these biomolecules are sufficient to monitor pain intensities; 2) compare the difference of those biomarkers during pain assessment; 3) give recommendations about how these biomarkers should be used to evaluate pain intensities in differing patient groups. Most importantly, this review will be concentrated on acute pain, as chronic pain is more complicated which is 1) significantly affected by psychological and social factors; 2) associated with changes in pain threshold and central sensitization processes. Based on this point, this review is planned to concentrate on acute pain while subsequent research will further summarize how these biomarkers changes in the presence of chronic pain.

Materials and Methods

Pubmed, Web of Science and Medline were searched to obtain articles relevant to the topic area. The following keywords were used to search for articles: “acute pain ”, "plasma cortisol ", "saliva* cortisol", "serum cortisol", "blood cortisol"; “plasma substance P”, “acute pain” “saliva* substance P”, “serum substance P”, “blood substance P” and “acute pain ”, "plasma ATP ", "saliva* ATP", "serum ATP", "blood ATP".

Inclusion criteria were:

- - Study focus on the assessment of acute pain intensities
- - Was an original paper

Exclusion criteria were:

- - The articles which couldn’t be assessed via UCL electronic databases or UCL library.
- - The articles published in a language other than English
- - The articles published before 1990-01-01

Results

The procedure for reviewing and selecting articles is shown in Figure 4. 892 articles have been identified by the databases. Then 749 articles were identified after removing duplicates with Endnote X8. After screening for the abstracts, 17 articles were chosen for full-text reviewing. Then after excluding the articles not available English (n=2), not reported cortisol levels (n=2), not mentioned pain history (n=1), the type of pain is chronic pain (n=1), 11 articles have been reviewed.

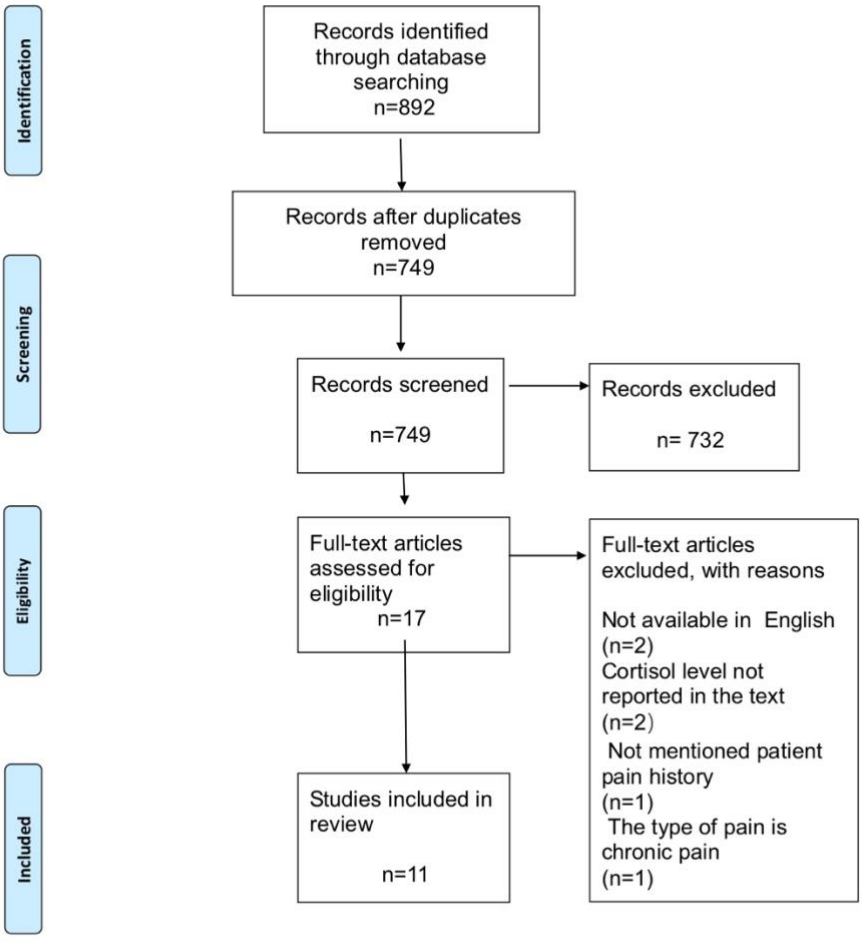

Figure 4: PRISMA flow gram for this review

1. Cortisol

1.1 Plasma cortisol in acute pain

Reference	Sample participants	Sample	Type of pain	Pain intensity assessment	Results
Esmat and Kassim, 2016	75 patients after surgery In three groups Age: TDF: 42.6 ± 4.6 TDM: 44.56 ± 3.4 Placebo: 43.56 ± 5.7 GENDER: TDF: F/M* 11/14 TDM: F/M 12/13 PLACEBO: F/M 10/15	Serum cortisol 2h after surgery	acute postoperative pain after lumbar laminectomy	VAS（0-10） TDF ： 1 TDM ： 2 Placebo group：5	 TDF：588.7 ± 91 nmol/L TDM：584 ± 107 nmol/L Placebo group：865.2 ± 128 nmol/L
Greisen et al., 2001	10 healthy volunteers Age:27 All males	Serum cortisol collected 9:30 am to 1:30 in an 15 minutes interval	Self-controlled painful electrical stimulation-induced acute pain	VAS（0-10） Before stimulation：0 After stimulation：8	 Before stimulation：331 ± 92 nmol/L Peak level after stimulation 10:30 am：445 ± 123 nmol/L
Edwards et al., 2008	42 healthy volunteers Age:43.8 ± 14.2 F/M 13/29	Serum cortisol collected at 2:35 PM	Cold pressor test	NRS（0-100） Before:0 Mean: 70.6 ± 24.1	Plasma cortisol Baseline: 226 nmol/l Peak ： 303.9 nmol/L

TDF: transdermal fentanyl delivery system; TDM: transdermal melatonin delivery system; F/M: Female/Male; VAS: Visual analogue scale ; NRS: numeric rating scale.

Figure 5: Results for plasma cortisol and acute pain

Esmat and Kassim randomly divide 75 patients into three groups: 25 patients receiving transdermal fentanyl (TDF) delivery system, 25 patients receiving transdermal melatonin (TDM) delivery system and 25 patients receiving placebo. They measure serum cortisol levels and VAS scores (0-10 scale) of post-operative pain 2 hours after surgery, and report that the VAS score is higher in placebo group than in TDF group and TDM group (averaging 5 vs. 1 vs. 2). Additionally, they also find that the serum cortisol level is higher in placebo group when comparing to other two groups (averaging 31.36±4.64 μg/dl vs. 21.34±3.3 μg/dl vs. 21.2±3.9 μg/dl, $P<0.01$) (Esmat and Kassim, 2016).

Greisen et al. use electric stimulation on 4 abdomen sites in turns to induce pain in 10 healthy volunteers, the pain intensity is 8 on an 0-10 VAS scale. Upon stimulation, serum

cortisol levels increases from 331±92 to 445±123 nmol/l in 30 minutes pose-stimulation and goes back to baseline level in 60 minutes-post awaking (Greisen et al., 2001).

Edwards et al. use cold pressor test to induce pain in 42 heathy subjects, patients are asked to immerse their right hands in the colder water at 4 ℃ for 30 seconds × 4 times with an interval of 2 minutes. Then in the fifth immersion, they are asked to remain until it reaches their pain endurance (<3min). The pain intensities are measured by 0-100 NRS rating scale. They report that the serum cortisol level increases and reach its peak (around 11ug/dl) at 15-min post-testing, and decreases slightly (<1 ug/dl) until 30 minutes post-testing, after which it decreases rapidly and returns to baseline level (around 8.5ug/dl) 60 minutes post-testing (Edwards et al., 2008).

1.2 Saliva cortisol in acute pain

Reference	Sample participants	Sample	Type of pain	Pain intensity assessment	Results
Goodin et al., 2012b	36 pain-free healthy volunteers Age:21; 47% Females	Saliva cortisol collected 5, 30, and 60 min after awakening	Cold pressor task	NRS(0-100) Subjects with higher pain intensities：72.1 Subjects with lower pain intensities: 50.5	Saliva cortisol AUG* Subjects with higher pain intensities: 33.6 Subjects with lower pain intensities: 23.7
Goodin et al., 2012a	10 pain-free healthy volunteers Age: 20.2 ±2.7; F/M：5/5	Saliva cortisol between 4:pm to 7:pm	Cold pressor task	NRS (0-100) Before:0 Mean during stimulation: 62.9±24.4	Saliva cortisol Baseline: 0.14mg/dl Peak : 0.2mg/dl
Karakoyunlu et al., 2019	51 pregnant women in the delivery room	Saliva cortisol during active phase and 4 hours after delivery	Acute Labor pain	VAS(0-10) Active phase: 9.21 ± 1.04 Postpartum:1.37 ± 0.60	 Active phase: 2.59±2.70 Postpartum: 2.22±1.81
Haug and Marthinussen, 2019	42 patients Age: 37.26 ± 12.37; F/M 17/25 39 healthy controls Age: 36.36 ± 10.86; F/M 22/17	Saliva cortisol in unstimulated saliva collected between 13:00 to 18:00	Acute dental pain	NRS(0-10) Patient: 7.0±2.59 Control:0	 Patient: 0.39±0.88 ug/dl Control: 0.14±0.11 ug/dl

AUG：area under the curve; VAS: Visual analogue scale ; NRS: numeric rating scale

Figure 6: Results for saliva cortisol and acute pain

Karakoyunlu et al. compare saliva cortisol levels in active phases (cervix dilated from 3 cm to 7 cm) and postpartum (4 hours after giving birth) phase of labor in 51 pregnant women, and report a higher level of cortisol along with a higher VAS score in active phase (Karakoyunlu et al., 2019).

A trend of increase of saliva cortisol after acute pain is also reported by Goodin et al, who use cold water pressor test to induce acute pain in 10 healthy volunteers, and report a positive relationship between NRS score and time-dependent increase in saliva cortisol ($r = .33$, $p = 0.04$) (Goodin et al., 2012a). Additionally, Goodin et al. also measure the concentration of saliva cortisol in 36 healthy subjects after cold water pressor task, and report that subjects with higher CAR AUG, which represent the cortisol output level during cortisol awaking response, have higher VAS ratings(Goodin et al., 2012b).

Haug and Marthinussen compare the saliva cortisol level in patients with acute dental pain to control group, and report a higher NRS score (7.0±2.59 vs. 0) along with a higher saliva cortisol level in patients group (0.39±0.88 ug/dl vs. 0.14±0.11 ug/dl) (Haug and Marthinussen, 2019).

2. Substance P

2.1 Plasma and saliva Substance P in acute pain

Reference	Sample participants	Sample	Type of pain	Pain intensity assessment	Results
Dalby et al., 1997	80 women in four groups* Age: Group 1*: 33.7 ± 1.4 Group 2*: 40.9 ± 1.3 Group 3*: 31.8 ± 1.2 Group 4*: 29.6 ± 1.5	Plasma SP Saliva SP	Acute Labor pain Acute postoperative pain after hysterectomy	Verbal Likert Pain Scale (LPS) (0-11) Group1: 0 Group2: 8 Group3: 0 Group4: 8	Plasma SP mg/ml Saliva SP mg/ml Group1: 7.18 ± 0.15 1.48 ± 0.21 Group2: 5.7 ± 0.19 2.15 ± 0.34 Group3: 6.54 ± 0.16 1.61 ± 0.23 Group4: 6.34 ± 0.12 1.92 ± 0.26
Lisowska et al., 2016	23 patients after total knee replacement(TKA) Age: 59±10 years F/M:22/1	Plasma SP	Acute postoperative wound pain after TKA	NPS(0-10) Immediately after surgery: 4 36h after surgery: 3	Plasma SP Immediately after surgery: 1200 pg/ml 36h after surgery: 200 pg/ml
Brandow et al., 2016	12 SCD* patients with acute pain Age：12.1 ± 3.5 F/M: 8:4 25 SCD patients without acute pain： Age: 11.18 ± 3.8 F/M:17/8	Plasma SP	Acute pain caused by SCD	Not measured	SCD with acute pain: 78.1 ± 43.4 pg/ml SCD without acute pain: 32.4 ± 11.6 pg/ml CON: 22.9±7.6 pg/ml

Group1:non-pregnant patients not in pain; Group2 :non-pregnant patients in pain; Group3 :pregnant patients not in pain; Group4: pregnant patients in pain; SCD: stickle cell disease; TKA: total knee replacement surgery.

Figure 7 : plasma and saliva substance P in acute pain

Dalby et al. 1997, compares saliva and plasma SP in saliva and plasma in 4 groups including non-pregnant women with no acute pain, non-pregnant women with acute pain after hysterectomy surgery, pregnant women with no acute pain and pregnant women experiencing acute labor pain, and reports that 1) peripheral SP concentration is not significantly associated with pain intensities and 2) plasma SP level and saliva SP level is not significantly related (Dalby et al., 1997).

Brandow et al. , in a recent study, measure plasma SP in patients with sickle cell disease (SCD), a disease characterized by the intermittent acute pain in children and chronic pain in adult patients (Brandow et al., 2016). They compare the plasma SP level in patients with

acute pain to baseline level of plasm SP in healthy individuals, and find that plasma SP in SCD patient with acute pain is significantly higher that in control group (figure 8).

This result is in line with Lisowski's research, who finds that serum SP level is positively related to the acute postoperative pain intensities ($r=0.504$, $p<0.05$) after total knee replacement surgery (TKA) (Lisowska et al., 2016).

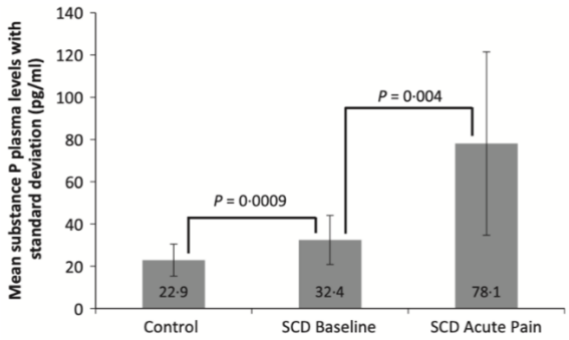

Figure 8 (Brandow et al., 2016): Plasma SP levels in healthy individuals, in SCD patient without acute pain and in SCD patient with acute pain. SCD: sickle cell disease.

3. ATP concentration in acute pain

Kumahashi et al. measure serum ATP in osteoarthritis (OA) patients with knee pain before and after treatment (706.2 ± 198 and 639.1 ± 205.7 μM) and report a decrease of serum ATP and VAS scores after treatment. Additionally, the serum ATP in OA patient is higher than that in the control group without knee pain. However, they don't find a significant correlation between change in serum ATP and change in VAS scores. Instead, a significant correlation between total ATP and change in VAS scores is demonstrated in their research(r = 0.56, p = 0.0032) (Kumahashi et al., 2011).

Discussion

1.Cortisol

1.1 Plasma and saliva cortisol in acute pain

Based on what is mentioned above in the introduction part, it can be seen that, in acute pain procedure, the cortisol level will increase and go back to baseline after some time. An increase of plasma cortisol followed by a rapid return to baseline is reported by three studies, suggesting plasma cortisol has the potential to reflect acute pain intensities.

Similarly, an increase of saliva cortisol along with increased pain intensities is reported in all four studies. In fact, the saliva cortisol has been shown to closely associated with plasma cortisol. Previous infusion study shows that the plasma cortisol transfers to saliva in 5 minutes (Cetinkaya et al., 2007). Additionally, various studies have shown a significant correlation between serum cortisol and saliva cortisol in both adults and infants (Matsukura et al., 2012, Cetinkaya et al., 2007).

Moreover, comparing to plasma cortisol, saliva cortisol has been demonstrated to have several advantages. Firstly, saliva cortisol is reported to be proportional to the unbound plasma cortisol, thus will not be influenced by cortisol-binding globulin(CBG) (Schmidt, 1997). Secondly, compared to plasma cortisol, the saliva cortisol can be collected in a more comfortable and stress-free way. Given that stress is an important factor to increase cortisol level, a stress-free way to collect samples is rather essential to assess pain levels accurately and subjectively.

However, one of the limitations of these studies is that the type of acute pain is limited, 3 of 7 studies used cold water presser task to induce acute pain in healthy volunteers. Thus to furtherly test the potential of using plasma and saliva cortisol to assess pain intensities,

future studies are expected to test cortisol changes in more acute pain models such as acute pain caused by heat stimulus, mechanical stimulus and chemical stimulus.

1.2 Difference in the baseline levels of cortisol

From the result, it can be noted that the cortisol baseline level differs according to the ages. In healthy volunteers, the highest cortisol level is observed in the volunteers aging 27 (Greisen et al., 2001), followed by the volunteers aging 43.8±14.2 (Edwards et al., 2008). The result is not in agreement with the current literature as an age-related increase in plasma cortisol levels have been shown in the studies(Seeman et al., 2001, Larsson et al., 2009, Nicolson et al., 1997).

One reason might be the different time to collect samples, as the cortisol concentration in the morning is higher than that in the afternoon, which is related to the circadian rhythm of cortisol. Typically, cortisol reaches at its peak 30 minutes post-awaking, followed by a steady decline and reaches at its lowest level in the midnight (Legler et al., 1982). This circadian rhythm is regulated by the suprachiasmatic nucleus (SCN) (Leproult et al., 2001), a pacemaker in the hypothalamus containing about 10000 neurons to receive signals from retina cells (Rollag et al., 2003). In the morning, the retinal cells will be activated by light, and send neural messages to SCN clock cells and CLOCK genes, which eventually drive signals outside to HPA-axis and control the releasing of cortisol(Meijer and Schwartz, 2003). Then the cortisol will be released and provides sufficient energy for daily activities via regulating glucose utilization, stimulating lipolysis, and regulating the protein breakdown and synthesis (McMurray and Hackney, 2005, Heim et al., 2000, Brillon et al., 1995).

Based on the releasing pattern of cortisol, when considering to use cortisol as a biomarker to monitor acute pain intensities, the baseline level of cortisol should be set in a 24-h pattern, as different collection time could result in a difference in cortisol level. As well as this, cortisol

might not be suitable to assess acute pain intensities in infants as it remains unclear when is the emerging time of circadian rhythm in infants, with results in current studies varying from 3 months to 9 months (Price et al., 1983, Gunnar et al., 1996, Kiess et al., 1995). However, recent research reports stability in the baseline salivary cortisol levels in the late morning (11:00 am) in first-year infants, which fluctuate from 4.4 to 25 nmol/l in 90% cases (Tollenaar et al., 2010). This finding suggests the potential to use morning basal salivary cortisol levels to assess acute pain intensities in infants.

Another reason for the difference in age-related trend in the results with literature might be the difference in the gender percentage in these three studies. There were all males in the study with the highest plasma baseline level (Greisen et al., 2001) while studies have been shown that men have a higher baseline level of plasma cortisol than the age-matched women (Van Cauter et al., 1996). However, this difference is also reported to not be significant after menopause (Van Cauter et al., 1996), which suggests that cortisol baseline level is influenced by sex hormones. Thus, when considering baseline levels of cortisol, in addition to age and sample collecting time, sex hormones-related factors should also be taken into account. For instance, the plasma and saliva cortisol baselines in different stages in puberty, in different period of the menstrual cycle, in pregnant or non-pregnant women, in women before and after menopause will differ.

1.3 Challenge in using plasma and saliva cortisol as a biomarker to monitor acute pain

In addition to exploring different baselines influenced by various factors including age, gender, sex-hormone levels and sample collecting time, one of the biggest challenges to use plasma and saliva cortisol as a biomarker to assess acute pain intensities is to distinguish the pain-related cortisol increase and stress-related cortisol increase.

In 7 studies related to cortisol, 4 studies considered the stress response of cortisol. Edwards et al. excluded the influence of psychological factors on cortisol levels as they measured the pain catastrophizing levels after pain procedures and reported that catastrophizing was not related to cortisol reactivity (Edwards et al., 2008). Haug and Marthinussen evaluated the stress level of their participants in their research with a simple questionaire, and reported that no patient reported an acute pain-related stress in their study (Haug and Marthinussen, 2019). Goodin et al., adopted the perceived stress scale (PSS) to assess stress level and concluded that CAR might be an indicator of stress (Goodin et al., 2012a). Karakoyunlu et al. assessed the stress level via PSS and demonstrated a correlation between cortisol level and stress level(Karakoyunlu et al., 2019).

From those results, it can be seen that it's hard to monitor or exclude the stress response of cortisol in acute pain procedure. In fact, as acute stress such as fear and tension is part of the consequence of acute pain, there's no need to exclude them all to asses pain intensities. The point is to distinguish the stress-only induced cortisol increase and pain-induced cortisol increase. For instance, experiments can be carried out to compare the fear-only caused increase of plasma cortisol and fear + acute pain caused increase of plasma cortisol. Additionally, although it's hard to exclude the impact of acute stress, it's possible to monitor the impact of chronic stress such as depression on cortisol level. For instance, it's important to collect the chronic stress history of patients such as psychological disease, chronic disease and social stress. Accordingly, different baselines of cortisol in terms of different chronic stress should be set to monitor the pain-related cortisol changes.

To summarize, both the plasma and saliva cortisol have the potential to monitor pain intensities in acute pain. However, as cortisol is a hormone associated to stress response, how to distinguish of the change caused by stress such as fear, tension and depression from that of pain could be a challenge in the future study. Furthermore, the baseline of plasma and saliva cortisol also differ in different ages, thus when considering to use the cortisol as a

biomarker, it is of great importance to explore different baseline levels based on different ages and genders. Furthermore, the cortisol baselines should be set in a 24-h pattern as cortisol is released in circadian rhythm, and sample collection time can result in a difference in cortisol levels. Additionally, the cortisol might not be suitable to assess pain intensities in infants due to the unstable releasing pattern of cortisol (Lewis and Thomas, 1990, Gunnar et al., 1996) caused by in-mature HPA-axis in infants, although the potential to use morning saliva cortisol level at 11:00 am to assess pain intensities is reported (Tollenaar et al., 2010).

Last but not least, future studies are also recommended to test the changes of plasma and saliva cortisol in more types of acute pain models as current literature mainly concentrating on the cold-presser-task induced acute pain.

2. Substance P

2.1 Plasma SP in acute pain

An increasing level of plasma SP along with higher acute pain intensities are observed in two studies. However, no association between plasma SP and acute pain intensities is observed in Dalby's research. This might be due to that they recruited both pregnant and non-pregnant subjects, who had different hormone levels. Given that they didn't consider the impact of hormones on the plasma SP, the result is not convincing. In fact, research shows that in pregnancy, pain threshold increases due to the higher concentration of sex hormones(Gintzler, 1980, Blomqvist, 2000). This explains the result that the plasma SP level in the pregnant group (group 4) is higher than that in non-pregnant women (group 2) although they report same level of pain intensities. Additionally, the average age of four groups is not matching, as current studies remain controversial when it comes to the age-related changes in SP (discussed in 2.3), the result needs further exploration.

Interestingly, Brandow et al. also report a higher plasma SP baseline level in SCD patient without acute pain when comparing to healthy individuals, which might be related to chronic

pain states in SCD patient. This is in line with the previous studies report that SP is also involved in the process of neural sensitization(Jang et al., 2011). However, the limitation of this study is that the mean age of SCD patient without acute pain is lower than the mean age of healthy control group (12 vs.21.5), thus whether age would have an impact on the result requires further research.

2.2 Saliva SP in acute pain

For saliva SP, only Dalby's research reports an increase of saliva SP in patients with higher pain intensities but the result is not significant (p>005). However, based on the age and sex-hormones factors mentioned above, the result is not convincing either. Additionally, current literature have studied the correlation between plasma SP and saliva SP but the results remain controversial. Jang et al. report that there is a significant positive relationship between saliva SP and plasma SP ($r=0.579$, $P<0.05$) (Jang et al., 2011). However, no significant correlation is observed in recent research (Jasim et al., 2018). Consequently, it's hard to conclude whether the saliva SP is sufficient to monitor acute pain intensities.

Furthermore, for saliva SP, the sample collection methods could also influence the result of SP concentration. Jasim and his colleagues compare the SP concentration in unstimulated whole saliva (UWS), unstimulated sublingual saliva (USS), stimulated parotid saliva (SPS), stimulated sublingual saliva (SSS) and stimulated whole saliva (SWS) in healthy subjects, and report that the highest concentration of saliva SP is measured in SSS, followed by UWS, SWS and SPS (370 ± 185 vs. 257 ± 89 vs. 23 ± 27 vs.11 ± 17 pg/ml, $p<0.01$) (Jasim et al., 2018). Thus future research is recommended to take the saliva collection methods into account when designing the trials to explore the correlation between saliva SP and acute pain intensities.

2.3 Challenge in using plasma and saliva SP as a biomarker to monitor acute pain

The biggest challenge to use SP as a biomarker to monitor acute pain is that the baseline levels of plasma and saliva SP remain unclear in current literature.

O'Dorisio et al.analyze blood SP levels in 41 healthy children aging from 1 month to 21 years old, and report the blood substance P in 0-11 months, 12-35 months, 3-5 years, 6-11 years and 12-21 years old group are 81±7, 94±49, 108±50, 110±65.2 and 55±38.4 respectively (O'Dorisio et al., 2002). In contrast, several studies report there are no age-related changes in the plasma SP concentration (Kunt et al., 2000, Deuschle et al., 2005).

Based on those studies, it can be seen that the baseline level of plasma SP remains unclear in current literature. However, to explore the possibility to use SP as a biomarker to assess acute pain intensities, it is essential to classify the baseline levels in different ages and genders. Consequently, there need more studies focusing on the age-related and gender-related in the changes of plasma SP baseline level.

To summarize, plasma SP has the potential to assess pain intensities in acute pain. However, more studies are required in the future to study the difference in plasma SP baselines in different ages and genders. For saliva SP, it is difficult to draw a conclusion due to the lack of existing evidence. More researches are required in this area and further studies are recommended to emphasize on the difference in saliva collecting method, as it can result in the difference in the measured SP concentration.

3. ATP concentration in acute pain

Only one study was found to focus on the relationship between plasma ATP and acute pain intensities thus it is hard to hypothesis whether ATP is sufficient to monitor pain intensities. In previous study, Hamilton and his colleagues apply iontophoresis to deliver ATP to the forearm skin of 12 healthy volunteers for 4 minutes, and find that the pain intensities

increased with the duration of ATP application and decrease rapidly after the termination of iontophoresis(Appendix 6) (Hamilton et al., 2000). This suggests the ATP-mediated pain is dose-related thus there might be a correlation between ATP concentration and acute pain intensities.

Moreover, in addition to ATP, various literature has been focusing on the role of P2X receptors in pain, In previous research, for instance, increased peripheral injection of P2X receptors agonists have been shown to increase pain sensation and pain responses in rat models(Bland-Ward and Humphrey, 1997). However, subsequent research has demonstrated that P2X receptor antagonists evoke no differences in C fibers nociception at dorsal horn level (Stanfa et al., 2000). Additionally, ATP receptors are only demonstrated to be associated with acute pain related to tissue injury and inflammation but not response to acute mechanical stimuli. This is due to that P2X3-null rat shows no difference in the behavioral responses towards acute stimuli (Souslova et al., 2000). Based on those findings, P2X3- mediated nociception is more relevant to inflammatory acute pain, and therefore ATP might be more suitable to detect acute pain evoked by inflammation or tissue injury.

Additionally, another point to support the potential to use ATP as a biomarker of acute pain intensities is that ATP has also been demonstrated to be associated with other neurotransmitters such as glutamate and substance P (Illes et al., 2001, Tsuda et al., 1999). Similar to SP, glutamate is one of the most essential excitatory neurotransmitters within the central nervous system (CNS) (Danbolt, 2001). P2X receptors on presynaptic neuron facilitate the release of glutamate while adenosine, the degradation product of ATP, inhibits the release of glutamate from presynaptic neuron (Illes et al., 2001).

Moreover, research reports that an increased level of glutamate could increase the peak level of astrocytes-released ATP by two times in rat models at spinal cord, which becomes seven times when glutamate is co-applied with SP (Werry et al., 2006). They furtherly report

that the reason might be that glutamate alone could increase ATP release through α-amino-AMPA receptors (Appendix 7) while co-application of glutamate and SP could also stimulate NMDA and metabotropic receptors (Werry et al., 2006). Moreover, as plasma and saliva glutamate concentration have been demonstrated to be associated with chronic pain intensities (Tripathi et al., 2018, Gerdle et al., 2014, Ferrari et al., 2009, Kawaguchi et al., 2015, Wesseldijk et al., 2008), ATP also has the potential to influence chronic pain intensities via interaction with glutamate.

As well as this, Park et al. recently demonstrate that the impact of SP on ATP is through the stimulation of NK1 receptors. They find that P2X3 receptors and NK-1 receptors are co-located on small-sized peripheral neurons, and therefore the activation of NK-1 receptors could in turns stimulate P2X3 receptors, increasing ATP-mediated nociception (Park et al., 2010).

To summarize, few studies report how ATP concentration changes in acute pain. However, various literatures have reported the P2X3 mediated nociceptive pain pathways. Additionally, research shows that P2X3 is more involved in acute pain caused by inflammation and tissue injury, thus ATP might be an ideal choice to detect pain accompanied by inflammation.

Furthermore, ATP is shown to be closely related to glutamate, SP and their receptors. Thus further researches are also required to study the correlation of ATP and these two biomarkers under the presence of acute pain at both peripheral nerves and the central nerve system. If the correlation is significant, then ATP will have a higher potential to be adopted as a biomarker of acute pain, given that it can also present changes in other two essential neurotransmitters that are vital in acute pain.

Limitations

The greatest limitation of this review is the small number of available studies related to the topic area. Only 11 papers have been identified, while 7 of the studies are related to plasma and saliva cortisol, three are relevant to plasma and saliva SP, and one is related to plasma ATP.

Conclusion

To summarize, there is sufficient evidence to support the potential of using plasma and saliva cortisol as a biomarker to monitor acute pain while more research is required to distinguish acute pain-caused cortisol increase and stress caused cortisol increase. Additionally, more work is recommended to be conducted to explore different baseline levels of plasma and cortisol baselines in different ages and genders in a 24-H pattern. As well as this, the baseline level of cortisol is also affected by the sample collecting time, as cortisol is released in a circadian pattern. Consequently, cortisol is not suitable to assess pain intensities in infants due to the unstable releasing pattern and immature HPA-axis in infants. But the cortisol may be appropriate to assess pain intensities in both older patients with cognitive impairments and unconscious patients in ICU as the age-related and gender-related changes in cortisol baseline levels have been widely studied.

For SP, in spite of small numbers of relevant studies, plasma SP has the potential to work as a biomarker to assess acute pain intensities. Especially in the situation when it is hard to evaluate the impact of chronic stress (e.g. psychological factors, social factors and chronic disease) on cortisol changes, plasma could be adopted as a supplement to cortisol to assess acute pain intensities. However, there is a lack of literature focusing on the baseline difference upon genders and ages of plasma SP, thus more research is required in this area. Additionally, there is a lack of evidence to support the potential to use saliva SP as a biomarker to monitor acute pain intensities. More studies are required in this area, and future

studies are recommended to take the different saliva collecting methods to into account, which might lead to different SP concentration.

Only one study is relevant to ATP, thus it is hard to conclude whether the ATP is suitable to be adopted as a biomarker to assess acute pain intensities. However, various studies show that ATP-P2X3 pain pathway is involved in acute inflammation pain, suggesting a potential to use ATP as a monitor for acute pain intensities related to inflammation or potential tissue damage.

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Appendices:

1. Pain assessment tools in elders with cognitive impairment (Herr, 2011):

Tool Name	References
The Abbey Pain Scale (Abbey)	Abbey J, Piller N, De Bellis A, et al: The Abbey pain scale: A 1-minute numerical indicator for people with end-stage dementia. Int J Palliat Nurs 10:6-13, 2004
Checklist of Nonverbal Pain Indicators (CNPI)	Feldt KS, Ryden MB, Miles S: Treatment of pain in cognitively impaired compared with cognitively intact older patients with hip-fracture. J Am Geriatr Soc 46:1079-1085, 1998
Certified Nurse Assistant Pain Assessment Tool (CPAT)	Cervo FA, Raggi RP, Bright-Long LE, et al: Use of the certified nursing assistant pain assessment tool (CPAT) in nursing home residents with dementia. Am J Alzheimers Dis Other Demen 22:112-119, 2007
Discomfort Behavior Scale (DBS)	Stevenson KM, Brown RL, Dahl JL, et al: The Discomfort Behavior Scale: A measure of discomfort in the cognitively impaired based on the Minimum Data Set 2.0. Res Nurs Health 29:576-587, 2006
Disability Distress Assessment Tool (Dis DAT)	Regnard C, Reynolds J, Watson B, et al: Understanding distress in people with severe communication difficulties: Developing and assessing the Disability Distress Assessment Tool (DisDAT). J Intellect Disabil Res 51(Pt 4):277-292, 2007
The Doloplus 2 (Doloplus-2)	Holen JC, Saltvedt I, Fayers PM, et al: Doloplus-2, a valid tool for behavioural pain assessment? BMC Geriatr 7:29, 2007
Elderly Pain Caring Assessment 2 (EPCA-2)	Morello R, Jean A, Alix M, et al: A scale to measure pain in non-verbally communicating older patients: The EPCA-2 Study of its psychometric properties. Pain 133:87-98, 2007
Mobilization-Observation- Behavior-Intensity-Dementia Pain Scale (MOBID)	Husebo BS, Strand LI, Moe-Nilssen R, et al: Mobilization-Observation-Behavior-Intensity-Dementia Pain Scale (MOBID): Development and validation of a nurse-administered pain assessment tool for use in dementia. J Pain Symptom Manage 34:67-80, 2007
Nursing Assistant-Administered Instrument to Assess Pain in Demented Individuals (NOPPAIN)	Snow AL, Weber JB, O'Malley KJ, et al: NOPPAIN: A nursing assistant-administered pain assessment instrument for use in dementia. Dement Geriatr Cogn Disord 17:240-246, 2004
The Pain Assessment Checklist for Seniors with Limited Ability to Communicate (PACSLAC)	Fuchs-Lacelle S, Hadjistavropoulos T: Development and preliminary validation of the pain assessment checklist for seniors with limited ability to communicate (PACSLAC). Pain Manag Nurs 5:37-49, 2004
Pain Assessment for the Dementing Elderly (PADE)	Villanueva MR, Smith TL, Erickson JS, et al: Pain Assessment for the Dementing Elderly (PADE): Reliability and validity of a new measure. J Am Med Dir Assoc 4:1-8, 2003
The Pain Assessment in Advanced Dementia Scale (PAINAD)	Warden V, Hurley AC, Volicer L: Development and psychometric evaluation of the Pain Assessment in Advanced Dementia (PAINAD) scale. J Am Med Dir Assoc 4:9-15, 2003
Pain Assessment in Noncommunicative Elderly Persons (PAINE)	Cohen-Mansfield J. Pain assessment in noncommunicative elderly persons–PAINE. Clin J Pain 22:569-575, 2006
Pain Behaviors for Osteoarthritis Instrument for Cognitively Impaired Elders (PBOICIE)	Tsai PF, Beck C, Richards KC, et al: The Pain Behaviors for Osteoarthritis Instrument for Cognitively Impaired Elders (PBOICIE). Res Gerontol Nurs 1:116-122, 2008
Rotterdam Elderly Pain Observation Scale (REPOS)	Van Herk R, van Dijk M, Tibboel D, Baar FPM, de Wit R, & Duivenvoorde HJ. The Rotterdam Elderly Pain Observation Scale (REPOS): A new behavioral pain scale for noncommunicative adults and cognitive impaired elderly. J Pain Man 1:357-366, 2009

2. Behavioral indicators for patients in ICU (Roulin and Ramelet, 2012).

Behavior	Examples
Facial expressions	Slight frown, sad, frightened face
	Grimacing, wrinkled forehead, closed or tightened eyes
	Any distorted expression
	Rapid blinking
Verbalizations, vocalizations	Sighing, moaning, groaning
	Grunting, chanting, calling out
	Noisy breathing
	Asking for help
	Verbal abusiveness
Body movements	Rigid, tense body posture, guarding
	Fidgeting
	Increased pacing, rocking
	Restricted movement
	Gait or mobility changes
Changes in interpersonal interactions	Aggressive, combative, resists care
	Decreased social interactions
	Socially inappropriate, disruptive
	Withdrawn
Changes in activity patterns or routines	Refusing food, appetite change
	Increase in rest periods
	Sleep, rest pattern changes
	Sudden cessation of common routines
	Increased wandering
Mental status changes	Crying or tears
	Increased confusion
	Irritability or distress

3. Self-report assessment tools in children (Brand and Al-Rais, 2019)

Characteristics of frequently used self-reporting pain assessment tools

Scale	Components	Age Range	Pros	Cons	Comments
Wong-Baker FACES	6 faces (0–5) Value 0-10	3–18 years	Easy, quick	Confusion with 'happiness'	Requires paper scale[a]
Faces Pain Scale Revised	6 mature faces (0–5) Value 0-10	4–12 years	Easy, quick	Confusion with 'happiness'	Requires paper scale[a]
Pieces of Hurt	5 stones or poker chips	3–8 years	Simple	Time consuming	Requires pieces[a]
Multiple-sized Poker Chip	4 poker chips increasing in size	4–6 years	Simple	Time consuming	Requires chips[a]
Numerical Analogue	Verbal scale 0–5 or 0-10	8–18 years	Easy, quick	Requires numeracy	No props required
Visual Analogue	10cm line Scale 0–5 or 0-10	8–18 years	Easy, quick, versatile	Requires proportionality	Requires pen & paper[a]
Adolescent Paediatric Pain Tool	Body map drawing & word graphic scale	8–18 years	Detailed	Time consuming	Requires pen & paper[a]

[a] Adjuncts may have cost, time and infection control implications.

4. FLACC pain assessment tool (Brand and Al-Rais, 2019)

Face, Legs, Activity, Cry, Consolability (FLACC) behavioural pain scale

Category	Score		
	0	1	2
Face	No particular expression or smile	Occasional grimace or frown, withdrawn, disinterested	Frequent to constant quivering chin, clenched jaw
Legs	Normal position or relaxed	Uneasy, restless, tense	Kicking, or legs drawn up
Activity	Lying quietly, normal position moves easily	Squirming, shifting back and forth, tense	Arched, rigid or jerking
Cry	No cry (awake or asleep)	Moans or whimpers, occasional complaint	Crying steadily, screams or sobs, frequent complaints
Consolability	Content, relaxed	Reassured by occasional touching hugging or being talked to, distractable	Difficult to console or comfort

Note: each of the five categories is scored between 0 and 2, resulting in a total score of 0–10.

5. Pain assessment tools in children with cognitive impairment (Hauer and Houtrow, 2017)

r-FLACC[18]
- Revised from the FLACC to include pain behaviors specific to children with cognitive impairment
- Like the FLACC, a 5-item pain assessment tool with a score ranging from 0 to 10
- Allows parents to individualize by adding behaviors specific to their child
- Option of indicating individualized behaviors can be beneficial for children with atypical pain behaviors and lack of other typical features, which may result in a false low score on other tools

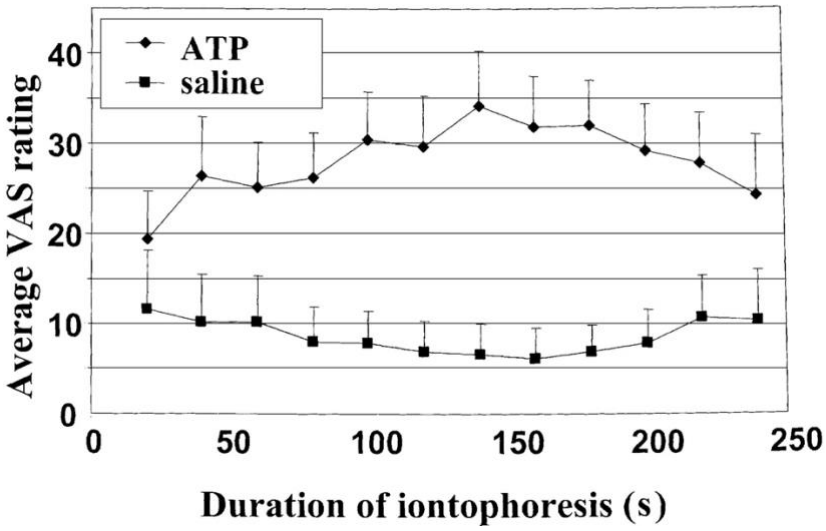
INRS[19]
- A personalized pain-assessment tool for nonverbal children with intellectual disability, based on the parent's knowledge of the child, developed for use in the hospital
- Parents and caregivers identify behaviors that indicate no pain to the worst possible pain on a scale ranging from 0 to 10
- Moderate to strong correlation between INRS ratings and NCCPC-PV (see below) total scores
- Option of indicating individualized behaviors can be beneficial for children with atypical pain behaviors and lack of other typical features, which may result in a false low score on other tools

NCCPC-PV[20]
- 27-item pain-assessment tool for children with severe cognitive impairment
- Moderate to severe pain determined at a cutoff of ≥11 of 81
- In Breau et al,[20] this cutoff provided a sensitivity of 0.88 and specificity of 0.81
- Available for download for clinical use or use in research funded by not-for-profit agencies at http://pediatric-pain.ca/resources/our-measures/

NCCPC-R[21]
- 30-item pain-assessment tool designed for nonverbal children ages 3–18 y with severe cognitive impairment
- Moderate to severe pain determined at a cutoff of ≥7 of 90
- In Breau et al,[21] this cutoff provided a sensitivity of 0.84 and specificity of 0.77
- Revised from the NCCPC-PV (postoperative version)
- Available for download for clinical use or use in research funded by not-for-profit agencies at http://pediatric-pain.ca/resources/our-measures/

ppp[10]
- A 20-item pain-assessment tool for children with severe to profound cognitive impairment
- Scores of ≥14 were generally associated, by observers, with moderate or severe pain
- A cutoff of 14 provided a sensitivity of 1.0 and specificity of 0.91
- The tool is arranged to provide different scores to indicate a rating for "on a good day," "most troublesome pain," "second-most troublesome pain," etc
- Available to download from the Web, after registration at www.ppprofile.org.uk

6. Duration of iontophoresis of ATP or saline on forearm skin and VAS ratings (Hamilton et al., 2000).



7. Glutamate receptors (Baldridge et al., 2011)