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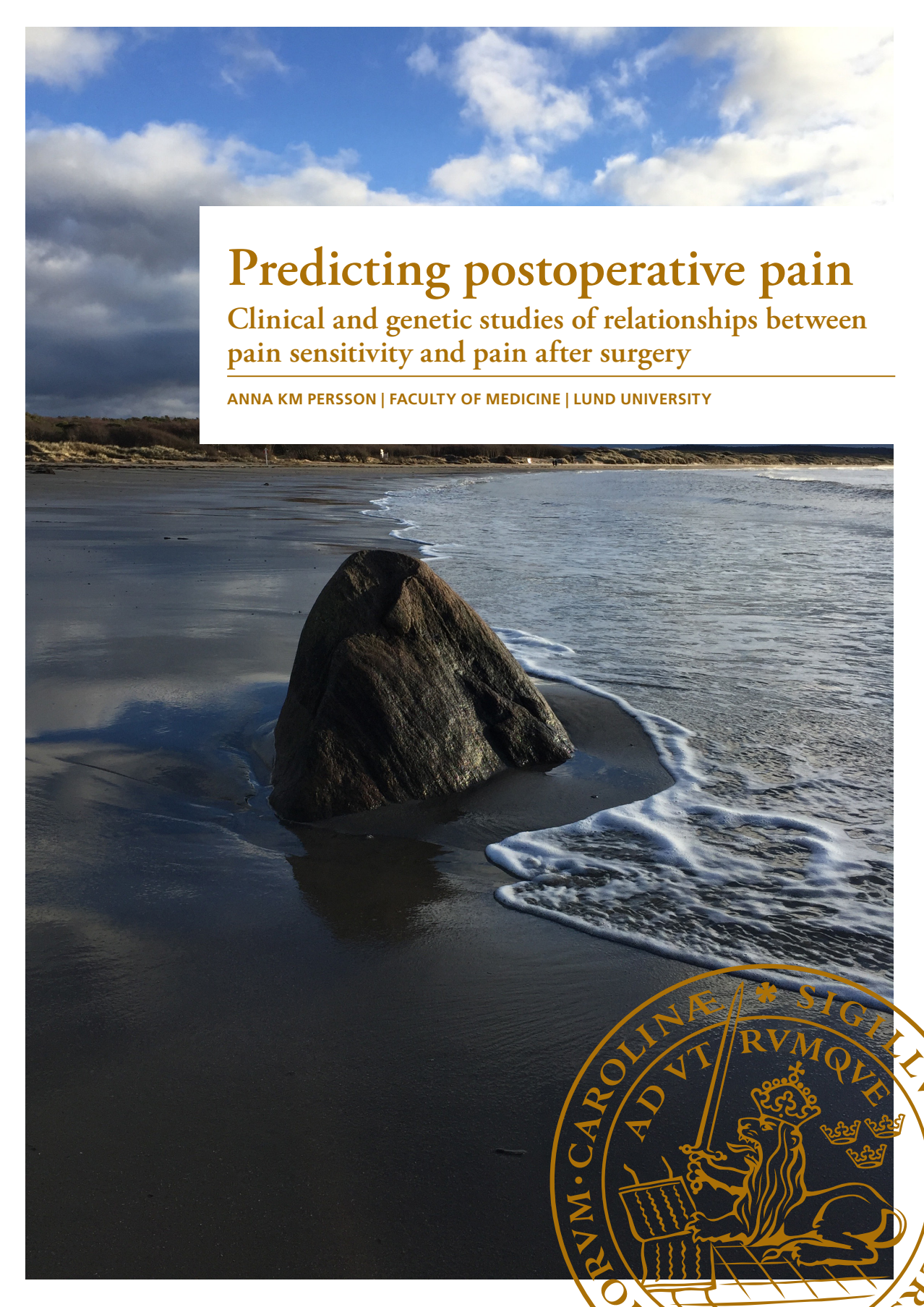
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Predicting postoperative pain

Clinical and genetic studies of relationships between pain sensitivity and pain after surgery

ANNA KM PERSSON | FACULTY OF MEDICINE | LUND UNIVERSITY



Predicting postoperative pain

Clinical and genetic studies of relationships
between pain sensitivity and pain after surgery

Anna KM Persson



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Department of Clinical Sciences Malmö

DOCTORAL DISSERTATION

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<p>Many patients experience pain after surgery. Postoperative pain may lead to delayed mobilization, persisting pain, and psychosocial distress. Others are given excessive analgesic doses and experience side effects. More optimal use of pain relief has several advantages, such as faster postoperative mobilization and fewer incidents of venous thromboembolism or infection. To achieve this, we must identify individuals at increased risk of severe postoperative pain, but simple and reliable techniques for prediction of postoperative pain have yet to be discovered.</p> <p>We know that individual factors such as female gender, low age, considerable pre-operative pain, and expectations on a painless postoperative course all increase the risk of severe postoperative pain. Studies have also shown that the estimation of pain thresholds with various modalities, such as heat, cold, pressure, or electricity, can be linked to pain intensity after surgery, but those tools are rarely used in clinical routine practice. To instead use painful components of routine preparation for surgery in order to evaluate individual pain sensitivity and predict postoperative pain would be almost revolutionary.</p> <p>This thesis (I-IV) was based on clinical studies designed to investigate whether painful procedures during routine preparation for surgery can be used to predict postoperative pain (I, IV), to test whether electrical pain threshold levels can be used to predict postoperative pain (II), and to identify possible genetic differences accountable for individual pain sensitivity (III).</p> <p>Painful routine procedures can be used for prediction of acute postoperative pain. Patients reporting pain intensity at or above 2.0 VAS units to be associated with peripheral venous cannulation were found to have 3.4 times higher risk of severe acute pain after laparoscopic cholecystectomy (I), and 1.7 times higher risk of severe postoperative pain after various surgical procedures (IV).</p> <p>Electrical pain threshold levels are reproducible, and the technique is well tolerated by patients. However, the method was found to be useful for prediction of postoperative pain only in women and not in men (II). Weak correlation with postoperative pain intensity, found here as well as previously, and high gender-dependency, considerably limit the clinical value of this technique for routine use in peri-operative practice.</p> <p>In our analysis of possible genetic contributions to individual pain sensitivity we found minor-allele single nucleotide polymorphisms in the ABCB1 and COMT genes to be more common in patients with higher pain sensitivity (III). These findings suggest a possible genetic contribution of those single nucleotide polymorphisms to individual pain sensitivity, however without reaching statistical significance, probably due to insufficient numbers of study patients.</p>			
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Predicting postoperative pain

Clinical and genetic studies of relationships
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Anna KM Persson



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“Jag vet att vad som helst kan hända när som helst,
det är därför jag är alldeles lugn”

- Muminmamman

To my family

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List of publications

This thesis is based on the following original publications, referred to in the text by their Roman numbers:

- I. Persson AK, Pettersson FD, Dyrehag LE, Åkeson J.
Prediction of postoperative pain from assessment of pain induced by venous cannulation and propofol infusion.
Acta Anaesthesiologica Scandinavica 2016; 60: 160-76.
- II. Persson AK, Dyrehag LE, Åkeson J.
Prediction of postoperative pain from electrical pain thresholds after laparoscopic cholecystectomy.
Clinical Journal of Pain 2017; 33: 126-31.
- III. Persson AK, Pettersson FD, Åkeson J.
Single nucleotide polymorphisms associated with pain sensitivity after laparoscopic cholecystectomy.
Pain Medicine 2017; doi 10.1093/pm/pnx164.
- IV. Persson AK, Åkeson J.
Prediction of postoperative pain from assessment of pain intensity associated with venous cannulation.
Submitted for publication.

Abbreviations

APAIS	Amsterdam preoperative anxiety and information scale
APOP	Acute postoperative pain
CPM	Conditioned pain modulation
DNA	Deoxyribonucleic acid
DNIC	Diffuse noxious inhibitory control
EPT	Electrical pain threshold
GWAS	Genome-wide association
HADS	Hospital anxiety and depression scale
IASP	International Association for the Study of Pain
ICU	Intensive care unit
NRS	Numeric pain rating scale
OPRM1	Opioid receptor mu 1
OR	Odds ratio
PACU	Post anesthesia care unit
PCS	Pain catastrophizing scale
PPSP	Persistent postsurgical pain
QST	Quantitative sensory testing
ROC	Receiver operating curve
SNP	Single nucleotide polymorphism
STAI	State-trait anxiety inventory
TRPA1	Transient receptor potential ion channel A1
VAS	Visual analogue scale
VCP	Venous cannulation pain – pain associated with venous cannulation
VRS	Verbal rating scale
WDR	Wide dynamic range neuron

Sammanfattning på svenska

Trots den snabba utvecklingen av moderna tekniker för narkos och bedövning upplever många patienter smärta efter kirurgi. God smärtlindring efter en operation kan påskynda återhämtningen och därmed minska risken för komplikationer som blodpropp eller infektion. För att uppnå detta måste vi bli bättre på att hitta individer med ökad risk för postoperativ smärta redan innan de sövs.

Sedan tidigare känner vi till att individuella faktorer som kvinnligt kön, låg ålder, en sjukhistoria innefattande långvarig smärta och förväntningar på ett smärtfritt efterförlopp ökar risken för postoperativ smärta. I forskningsstudier har man också kunnat visa att människors smärtröskelnivå vid stimulering med värme, kyla, tryck eller elektrisk ström har samband med deras smärtkänslighet efter operation. Detta undersöks sällan till vardags inom vården. Om man istället kunde bedöma patienters smärtkänslighet genom att utvärdera hur de upplever åtgärder som gör ont i samband med förberedelse inför operation, skulle detta snabbt kunna visa sig praktiskt användbart.

Inom ramen för detta avhandlingsarbete, som bygger på fyra vetenskapliga arbeten (I-IV), har vi undersökt om nålsättning och injektion i blodet av sömnmedlet propofol (båda kan upplevas som smärtsamma) skulle kunna användas för att uppskatta risken för postoperativ smärta (I, IV). Vi har även utvärderat om ett instrument som med elektrisk ström kan fastställa enskilda människors smärtröskel även skulle kunna användas för att förutse vilka som har ökad risk för smärta efter operation (II). Dessutom har vi undersökt variationer och mönster i arvsanlag som är kopplade till smärtkänslighet (III).

Första delen av studien (arbete I-III) bygger på en observationsstudie vid Hallands sjukhus, där vi följde 180 patienter som fick gallblåsan bortopererad med titthålskirurgi, så kallad laparoskopisk kolecystektomi. Förberedelse-, narkos- och uppvakningsrutiner standardiserades. Innan patienterna sövdes fick de på en mätsticka, visuell analog skala (VAS) graderad från 0.0 - 10.0, markera sina upplevelser av dels hur ont nålsättningen gjort, och dels hur ont injektionen av narkosmedlet (propofol) gjort. De fick även testa sin elektriska smärtröskel. Efter operationerna registrerades på motsvarande sätt hur ont patienterna hade, samt hur tidigt och i vilka doser starka smärtlindrande läkemedel (opioider) tillfördes på uppvakningsavdelningen.

De patienter som skattat smärtan vid nålsättning till mer än 2.0 VAS-enheter hade i genomsnitt också mer ont efter operationen. Risken för att dessa patienter skulle uppleva smärta efter operationen visade sig vara mer än tre gånger högre (I).

Vi följde sedan upp undersökningen med en studie på ytterligare 600 patienter som genomgick olika typer av operationer med olika narkos- och bedövningstekniker, och fann att vårt nålsättningstest sannolikt kan användas för att förutsäga risk för smärta efter i princip alla former av kirurgi (IV). Använder man testet oberoende av patient och typ av kirurgi, så har patienter med smärta över 2.0 VAS-enheter vid nålsättning nästan dubbelt så hög risk att få ont efter operationen.

Avseende elektrisk smärtröskel så kunde vi koppla låga tröskelvärden till ökad smärta efter gallblåsekirurgi, men testet visade sig bara användbart på kvinnor. Hos kvinnliga patienter med högre smärtekänslighet (lägre smärtröskel än flertalet kvinnor) var risken för postoperativ smärta mer än fyra gånger högre än annars, medan testet inte kunde användas för att på motsvarande sätt förutsäga risken för svår smärta hos manliga patienter (II).

Vid undersökning av arvsanlagen (generna) fann vi samband med några bestämda varianter av baspar i generna, som tidigare visats ha betydelse för att signalera smärta. Den variant som vi lyfter fram som viktigast i vår undersökning (III) finns i en gen som kallas ABCB1 och har en viktig funktion för transport av ämnen mellan blodet och hjärnan. De resultat vi kom fram till behöver dock bekräftas i fler undersökningar, eftersom de bygger på omfattande genetisk information från ett ganska litet antal patienter.

Sammanfattningsvis har vi visat, att man kan använda smärtskattning vid nålsättning för att redan före en operation hitta patienter med ökad risk att uppleva smärta efter operation (I, IV). Denna metod är enkel att använda och kräver ingen extra utbildning eller särskild utrustning. Elektrisk smärtröskelmätning är en annan metod för att mäta individuell smärtröskel. Den kräver särskild utrustning och kan tyvärr bara användas för att förutsäga risk för postoperativ smärta hos kvinnor (II). Det finns förändringar i baspar i gener kopplade till smärta som skulle kunna kopplas till ökad smärtekänslighet men resultaten bör bekräftas i ytterligare studier (III).

Background

What is pain?

The International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (1, 2). The first known descriptions of pain are from Hippocrates’ teachings already in 400 BC. Pain, as it is defined in our days, began with the formation of IASP in Seattle in 1973 and a task force of specialists put together to define the taxonomy in 1979 (3). This taxonomy has since then been updated regularly by different groups of experts. The definition mentioned above was last revised in 2008. It comes with an explaining note “The inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment. Pain is always subjective”.

A recent, functional, way to classify pain is in a temporal manner as acute pain or persistent. Persistent pain is defined as pain for 3 months or longer. While acute pain is usually caused by some sort of tissue damage, in persistent pain the stimulus is often no longer present and pain is instead entertained by an upregulated or sensitized pain signaling system. Why certain patients develop persistent pain is largely unknown, but risk factors like female gender, psychological factors, and in general higher sensitivity to pain suggests that patient specific factors have a role, such as perhaps genetic factors.

Pain can also be classified depending on cause as nociceptive, neuropathic, psychological or unknown. This might have implication for therapy, where for example anti-epileptic or antidepressant drugs are more effective than opioids in neuropathic pain (4, 5). Recently a new mechanistic descriptor of pain has been proposed and accepted by the IASP as nociplastic pain, defined as “pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain”. This descriptor is proposed to help describe clinically altered nociception, which might lead to persistent pain (6). This further encourages research to identify altered nociceptive function.



Pain according to Karin 6 years of age.

Physiology of pain processing

Nociceptive signaling starts with activation of free nerve-endings branched from the main axon of a nociceptive neuron. These nociceptive receptors have high thresholds, meaning that the stimulus has to be strong to induce activation. In contrast to mechanoreceptors, nociceptive receptors do not adapt to repeated stimuli, leading to continuous signaling if the painful stimulation persists (7).

Primary nociceptive neurons are either A δ - or C-fibers. A δ -fibers are thin myelinated fibers connecting to a few interneurons within a small area in the spinal dorsal horn, leading to distinct, localized perception of sensation. In contrast, C-fibers are unmyelinated and branch in a more diffuse manner in the dorsal horn, giving rise to sensation of pain hard to localize precisely, and usually described as dull (7).

These primary neurons enter the spinal cord and synapse on second-order neurons within the dorsal horn. Axons of these neurons then traverse to the opposite side of the spinal cord and ascend within the spinothalamic tract to the thalamus. Some neurons alternatively ascend to the medulla oblongata, where they activate the

autonomous nervous system and may increase the heart rate, blood pressure, and respiratory rate. In the thalamus, the signal is relayed to tertiary neurons transmitting the nociceptive signal to the primary sensory cerebral cortex, making us aware of the pain. There are also tertiary neurons ascending from the thalamus to the limbic system, the insula, the anterior cingulate cortex, the amygdala and other subcortical areas, triggering emotional responses (4, 8, 9).

Inflammatory mediators in the periphery may activate primary neurons in response to repeated injury or inflammation. This causes them to lower their activation threshold, thereby being easier to activate with higher responses to stimulation or even spontaneous signaling. This is called primary sensitization and may give rise to primary hyperalgesia. This process can also cause allodynia, where normally non-painful stimuli suddenly become painful. Dysregulation of this response may promote transition to persisting pain (10).

Similar mechanisms occur in the spinal dorsal horn, where interneurons, called wide-dynamic-range (WDR) neurons, are activated by repeated stimuli or inflammatory mediators. As these neurons normally also transmit non-nociceptive signals from larger body regions, upregulation (wind-up) will cause hyperalgesia within a larger area surrounding the site of injury or even induce allodynia. This is called central sensitization or secondary hyperalgesia (10).

We also have descending inhibitory systems. They exert their effects primarily within the dorsal horn by releasing monoamine-serotonin, norepinephrine, and dopamine. This phenomenon of endogenous analgesia is mediated mainly via cannabinoid and opioid signaling in the periaqueductal grey of the midbrain (11). Dysregulation of the descending inhibitory system may also explain transition of acute pain to persisting (10).

Assessment of acute pain

Pain is per definition subjective, and the only way to measure it is to ask the patient or make an assessment based on non-verbal communication like facial expression or other physical signs of pain. Acute pain should be evaluated by scoring of pain intensity, and there is consensus to use so called one-dimensional tools for evaluation. The tools most commonly used are the numeric rating scale (NRS), the verbal rating scale (VRS), and the visual analogue scale (VAS), and in recent years the VAS is the one most frequently used in clinical practice and research (12).

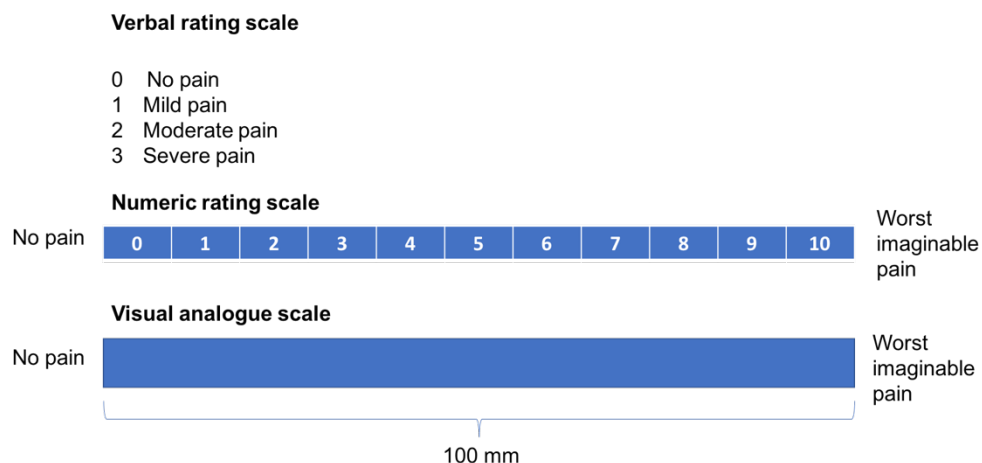


Figure 1. One-dimensional tools for evaluation of pain intensity
The most commonly used pain rating scales.

Verbal rating scale

The VRS consists of five words and corresponding numbers with which the patient can describe their pain. The scale was developed to easily evaluate success of treatment. It is short, simple and easy to use in clinical practice, but lacks accuracy for research with its few categorical words: ‘no pain’, ‘mild pain’, ‘moderate pain’, and ‘severe pain’ (13). It has however been shown to deliver reliable scientific information despite its simplicity (14).

Numeric rating scale

The NRS was first described in 1978 and consists of a line from 0-10 oriented either vertically or horizontally. The patient is asked to grade the pain intensity on a scale from 0 to 10, where 0 represents no pain and 10 the worst imaginable pain. Multiple versions of this scale now exist. The scale is easy to administer and easy to use. It can be delivered graphically or verbally (15). Relative to VAS this scale has shown high compliance, good responsiveness and ease of use (16). The scores on the two scales correspond well (17).

Visual analogue scale

The VAS is the tool mostly used to score pain intensity in clinical practice and clinical research. It consists of a vertical or horizontal line of 10 cm with 'no pain' at one end and 'worst imaginable pain' at the other. The patient is told to put a mark on the line corresponding to the actual intensity of pain, enabling the distance (in mm) to be exactly determined and used to score the pain intensity (15). A disadvantage in clinical practice is that it has to be used in two steps. First, the patient has to indicate the intensity on the scale, and then the investigator has to measure and record the response, and incorrect measuring is a potential cause of error (15).

Definitions and ratings

Studies differ on what is defined to be slight, moderate and severe pain on the VAS. When cancer patients were asked to define on the NRS pain intensity associated with mild, moderate or severe pain according to the VRS, the cut-off-points were set at 4 and 8 (18). Further attempts have been made to define corresponding levels and Hirschfeld et al. defined similar levels where slight pain was NRS lower than 4, moderate pain 4-7, and severe pain 8 and higher (19).

How big a change in pain intensity do you need to consider the improvement significant? According to Cepeda et al. this depends on the pain level. If the pain intensity at baseline is moderate a change in NRS of 1.3 is considered improvement, and at severe pain at baseline a change in NRS of 1.8 is needed to be considered an improvement (20). Bird et al. studied patients at the emergency department after trauma and found clinically significant changes in pain to be 1.3 VAS units in patients starting out with slight pain and 2.8 VAS units in patients with severe pain (21). Thus, clinically significant changes in pain are different along the length of the VAS. Others have used 50% pain reduction to be a significant reduction in pain (13).

Postoperative pain

Surgery causes tissue injury and release of histamine and inflammatory mediators inducing nociceptive signaling and experience of pain. Intense noxious input may cause peripheral and central sensitization. Approximately 80 % of patients who undergo surgery report acute postoperative pain, and less than half report adequate pain relief (22). In 10-50 % of postoperative patients the pain continues leading to persistent pain, with chronic physical disability and psychosocial distress in 2-10 % of these patients. Persistent postsurgical pain is defined as pain continuing for more than three months after surgery. Severe postoperative pain is believed to contribute to

conversion of acute to persisting postsurgical pain (PPSP) (23, 24). In a recent large study, APOP was found to be the most important risk factor for pain persisting beyond six months after surgery, with even higher odds ratios (OR) than pre-operative pain conditions (25). Patients identified before or soon after surgery to be at high risk for PPSP, for example those with severe APOP, should be referred to an acute pain service for early management and follow-up in an attempt to reduce the number of patients developing PPSP (26).

Uncontrolled acute pain after surgery comes with immediate and long-term complications. Once nociceptive signals reach the brain, the patient will be conscious of the pain, and simultaneous signaling to the limbic system will induce emotional responses. Neuro-endocrine stress responses will lead to activation of the hypothalamic-pituitary-adrenocortical and sympatho-adrenal systems with increased sympathetic tone, and higher plasma levels of catecholamines and catabolic hormones. The heart rate and blood pressure will increase as well as the oxygen consumption. The stress response with higher global demand for oxygen increases the respiratory work. Sympathetic activation also induces higher cardiac oxygen consumption, which, together with the general stress response, puts extra effort on the heart. The extent of stress response is proportional to the surgical trauma. This neuroendocrine response is believed to be a factor in development of a hypercoagulable state, which – together with inhibition of fibrinolysis, increased platelet reactivity and increased blood viscosity – may promote thrombo-embolism. Furthermore, secondary hyperglycemia may contribute to poor wound healing (5). Studies have indicated that, despite advances in pain prediction and treatment, postoperative pain remains insufficiently treated (27). Improved prevention of this important surgical complication is vital. Attenuation of pain and the stress response after surgery promotes early recovery and reduces the risks of a number of potentially harmful complications mentioned above (Fig. 2).

Pain sensitivity and quantitative sensory testing

Experimental pain can be evaluated with different modalities, where a painful stimulus is applied and the response quantified. Methods designed to test pain sensitivity are called quantitative sensory testing (QST). Pain from mechanical stimuli can be tested in a controlled manner by stimulation with touch, pinprick or pressure. Thermal stimulation with cold, heat or laser radiation is another modality. Finally, pain can also be induced by chemical substances, like capsaicin or mustard oil, and with electricity (as discussed in more detail below) (28).

Quantitative sensory testing can also mimic different processes in pain signaling. For example, applying a continuous cutaneous stimulus can cause primary hyperalgesia while a deeper stimulus, or intramuscular injection of a painful substance, is required

to induce secondary hyperalgesia. To mimic visceral pain the same stimuli as mentioned above can be applied inside hollow organs, like the gut. This has however, for obvious reasons, only been applied in experimental research (29).

Dynamic QST involves methods designed to evaluate more complex parts of pain processing. With these methods, wind-up can be tested through temporal and spatial summation with repeated stimuli. The descending inhibitory system or diffuse noxious inhibitory control (DNIC) can be tested with conditioned pain modulation (CPM). Using this method, initial application and evaluation of a painful test-stimulus is followed by the induction of pain at another site with another kind of stimulus. The test-stimulus is then re-applied and re-evaluated. The difference between the first and second evaluations of the painful test-stimulus reflects the level of DNIC. Less efficient DNIC is associated with more individual experience of pain (29).

More complex tests of dynamic QST such as DNIC efficacy (30) with CPM or temporal summation (31) are believed to more appropriately reflect the clinical situation where acute pain turns into persistent pain, and have also been shown to predict PPSP (32). For prediction of APOP these more complex methods are hard to apply in clinical practice, and they have not been found to be better than simple methods (33, 34).

Electrical pain thresholds

Among different methods for QST, testing electrical pain thresholds (EPT) is a technique that allows standardized timing and intensity when delivering the stimulus. The devices used to determine EPT levels are often handy and easy to use, and have been shown to be safe and reliable tools for measuring pain thresholds (35-38). The electrical stimulus is believed to bypass nociceptive receptors and directly activate neurons (28). The simplicity of the method and promising results in earlier studies (8, 14, 22) encourage further evaluation, as also proposed in recent reviews (39, 40). However, results concerning its predictive ability regarding postoperative pain are controversial. Levels of EPT have been shown to correlate with levels of acute postoperative pain after Caesarean section (35, 41). In contrast, studies in male patients have reported no such predictive properties of individual EPT levels (42-45). It has therefore been suggested that EPT, for unknown reasons, can only be used to predict postoperative pain intensity in women (39).

Higher nerve and receptor density in glabrous skin on the fingertips of women, together with a smaller area of stimulation, has been proposed to explain higher sensitivity to this kind of stimulation in women due to higher influx of electrical energy (46). The distance from the surface of the skin to the nerves may also influence the pain response (47).

Pain associated with venous cannulation

Peripheral venous cannulation, a procedure necessary in health care services, is many times associated with pain. The procedure has a success rate of 76-98 % and is, when successful, associated with pain levels of 2.5-3.0 VAS or NRS units (48-50). Multiple needle sticks induce more pain (48). The site of cannulation also influences the level of pain induced, and cannulation of the antecubital fossa is associated with less pain than the hand (51).

Although often considered painful, many health care professionals do not routinely offer pain relief. Reasons often stated are potential waste of time, doubt whether required, potential aggravation of cannulation, peer pressure, or practical difficulty. Pain associated with peripheral venous cannulation can be successfully prevented by topical application of eutectic mixture of local anesthetics (EMLA) or by infiltration of local anesthetic (50, 52).

As for the case with postoperative pain a positive correlation has been found between the level of anxiety before cannulation and the level of discomfort associated with the procedure (53). Suren et al. have shown that psychological factors like anxiety or pain catastrophizing might contribute to pain associated with venous cannulation, considering that pain catastrophizing score (PCS) levels correlate with pain associated with venous cannulation (54).

A recent obstetric study on associations between venous cannulation-induced pain (VCP) and pain during labor reported weak correlations between VCP and time to epidural request but no significant correlations with different measures of pain during labor (55).

Pain associated with propofol infusion

The drug most commonly used to induce general anesthesia is propofol.

Approximately 60 % of patients experience local pain on injection (56).

Traditionally, propofol has been considered to release pain-inducing kininogens upon contact with the vascular endothelium. Propofol has also been shown to activate vanilloid receptors, resulting in neuronal influx of calcium (56). Another theory is that pain is induced by activation of the transient receptor potential ion channel A1 (TRPA1) (57), but the exact mechanism responsible for pain upon intravenous injection of propofol remains unknown.

Many methods have been proposed to reduce the pain induced by administration of propofol. According to a recent review by Jalota et al., injection in the antecubital vein, and, especially if usage of the hand vein, pre-treatment of the vein with lidocaine in conjunction with venous occlusion, are the most efficient methods for reducing pain. A common present practice, to mix propofol with lidocaine before injection, is also effective but not as good as the other ones (56).

Remaining uncertainty regarding nociceptive mechanisms potentially involved makes peripheral intravenous infusion of propofol as a local pain-stimulus for predictive purposes questionable.

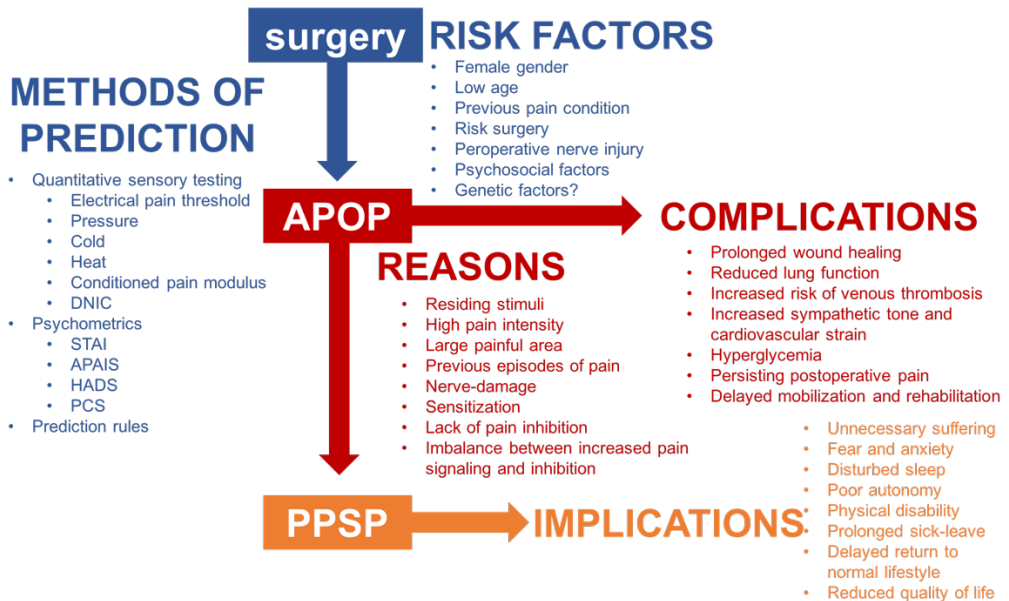


Figure 2. Proposed methods for prediction, and factors influencing development and complications, of acute (APOP) and persistent (PPSP) pain after surgery.

Prediction of postoperative pain

Known risk factors for postoperative pain are female gender, low age, pre-operative pain, psychosocial factors like anxiety and depression, certain types of surgery, especially if surgical nerve damage is caused, and large skin incision (23, 58). None of these factors are strong enough to be used alone for individual prediction of risk for severe postoperative pain. Pre-operative screening methods could potentially enable intensified attempts at prevention and treatment of pain in patients prone to pain after surgery. Several methods have been proposed to solve this important task (Fig 2).

Moderate to severe postoperative pain is still a problem in 20-40 % of surgical patients, and even everyday surgical procedures are often associated with considerable postoperative pain (59). Focus is currently shifting from procedure-specific towards

individualized therapeutic strategies to improve management of acute postoperative pain and reduce the risk for long-term transition to PPSP (60, 61).

Predicting risks for severe postoperative pain in specific patients is thus an important part of this strategy. Experimental tests have been reported to explain 4-54 % of interindividual variance in postoperative pain (39, 62), and psychological factors, in particular pain catastrophizing, are also important (63). Hence, widely considered, the way forwards is prediction models combining different methods (64).

Pre-operative pain score

Patient history of recurrent pain is the most important risk factor for higher levels of APOP, and probably also for development of PPSP (58, 65-67).

Several attempts have been made to evaluate individual pain sensitivity before surgery. However, there are only few examples of using pain experienced during procedures performed in the regular presurgical preparation – in contrast to QST – to predict postoperative pain intensity. Rago et al. reported, in 2012, that assessment of tourniquet-induced pain before surgery could predict postoperative pain intensity (68). Carvahlo et al. showed a correlation between VCP and time to epidural request when following 47 women in labor, suggesting a link between pain sensitivity and pain during labor (55). A few years later, Orbach-Zinger et al. published data suggesting associations between pain at injection of local anesthetics administered before spinal anesthesia during preparation for Caesarean section, and postoperative levels of pain intensity as well as analgesic requirements (69).

Psychometrics

Psychological factors may influence both acute and persistent pain. A number of specific questionnaires, like the state-trait anxiety inventory (STAI), the Amsterdam preoperative anxiety and information scale (APAIS), the pain catastrophizing score (PCS), and the hospital anxiety and depression scale (HADS), have all been reported to enable prediction of postoperative pain intensity levels (58, 70, 71).

The APAIS, a six-question questionnaire specifically designed to evaluate pre-operative anxiety score and information-seeking behavior (72), has been shown to improve prediction of postoperative pain in conjunction with other tools (58).

The HADS is a questionnaire designed to evaluate levels of anxiety and depression in somatic healthcare. It comprises a fourteen-item scale with seven items measuring anxiety and seven others measuring depression, providing a maximum score of 21 for each entity. A score of 0-7 is considered normal, whereas 8-10 indicate slight to moderate signs, and 11-21 assumed presence of mood disorder (73). The HADS has been used in research on pain prediction (61) and has been translated and validated in

Swedish (74). However, studies on prediction of postoperative pain from HADS scores have reported conflicting results. The scale has been used in several large studies looking at pre-operative factors and their ability to predict PPSP and has not been found to be significantly predictive (61) (75). No association between pre-operative HADS scores and VCP was found in a recent study in healthy volunteers (53).

The PCS comprises 13 questions measuring negative orientation around the thought of pain. It was first developed by Sullivan et al. in 1995 and has been frequently used and validated in pain research since then (76). The PCS is the psychometric tool showing the most promising results regarding predictability of acute postoperative pain (63, 71). It has been found to be associated with high pain experience with respect to both pain intensity and use of analgesics (61, 63, 70). The results are however conflicting, and other studies have shown no such predictive ability (24, 61). Its scores have also been found to have weak association with VCP. Suren et al. in 2013 presented an interesting study on pre-operative VCP measurements and PCS evaluations. The median pain intensity associated with venous cannulation on the back of the hand with a 20 G catheter was 3 NRS units. A weak correlation was found between PCS score and VCP ($r=0.197$, $p=0.011$). Patients with chronic pain scored higher, and females scored higher than males, on the PCS. Data on postoperative pain was unfortunately not reported (54).

Other factors closely linked to psychological factors have also been reported to affect pain sensitivity. Recently poor sleep quality was reported to be associated with higher pain intensity and use of analgesics after Caesarean section, and also with higher PCS scores (68).

To summarize the value of psychological analysis, in a meta-analysis, including 29 studies and 14 different psychometric evaluation questionnaires, only 55% of the included studies reported an association between increased preoperative anxiety or pain catastrophizing and PPSP. The pooled OR, based on 15 studies, however leaned towards an increased risk ranging from 1.55-2.10 (77).

Quantitative sensory testing

There is also research suggesting a link between pre-operative experimental pain thresholds and postoperative pain (39, 61, 63, 78-82). Various methods for estimating pain thresholds with different modalities of pre-operative QST, based on induction of different kinds of experimental pain, e. g. pain threshold to electrical stimuli (35, 43, 44) heat pain threshold, cold pain threshold (79, 80, 82-84) or pressure pain threshold (63, 81), have been reported to correlate with postoperative pain sensitivity. Quantitative sensory testing has been shown to predict up to 54 % of the variance in postoperative pain with better predictive strength than any other

known risk factor. Among different methods of QST, EPT has been reported to have the best predictive ability (39).

The predictive ability of QST is better than that of psychological factors, and combining them has in many studies not increased the predictive power (39). In a recent study however, reduced pressure pain threshold together with increased PCS was shown to be the best predictor of postoperative pain during movement 24 hours after surgery. The authors combined the two factors in a predictive model with a sensitivity of 71.4 and a specificity of 62.5 (63).

Prediction rules by combining risk factor evaluations

As no single factor or test alone has shown excellent, reproducible, results for prediction of postoperative pain, some researchers are shifting focus towards adding different factors to develop prediction rules. Already in 2003, Kalkman et al. suggested a preoperative prediction rule including age, gender, type of surgery, size of incision, preoperative pain scores, and APAIS scores, to predict severe postoperative pain with a sensitivity of 74 % and specificity of 61 % (58). This prediction rule was later validated and modified in another cohort (85).

In a large study designed to identify risk factors for PPSP after herniotomy, Aasvang et al. identified four factors associated with PPSP – pre-operative pain-related functional impairment, pre-operative pain-response to heat, intra-operative nerve injury, and postoperative pain intensity on day 30 (61). A predictive model was set up based on pre- and intra-operative factors (pain-related functional impairment, pain on heat QST, and surgical technique), and a risk plot presented as a prediction tool for postherniotomy pain with fairly good predictive ability (C-statistic 74 %).

Another study, including both clinical and genetic factors, found only two factors - pre-operative pain in the operating field, and other pre-operative pain – to be relevant in this context (75).

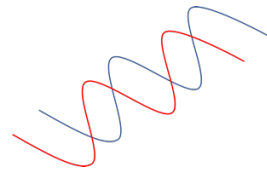
Recently another prediction model – based on a five-item risk index score, reflecting comorbid signs of stress, capacity overload in the past six months, pre-operative pain in the operating field, other pre-operative chronic pain, and movement-evoked pain five days after surgery – has been proposed to predict pain intensity six months after surgery (86) without having to test the patient in the immediate peri-operative period. Mathes et al. validated and updated this tool of prediction into a risk index for chronic pain (RICP) based on assessments of pre-operative pain in the operating field, movement-evoked pain five days after surgery, other pre-operative chronic pain, and female gender. The RICP was found to predict PPSP at 6 months after surgery with a sensitivity of 75 % and specificity of 73 % (25)

In theory, prediction tools are tempting, but so far, no proposed validated tool for prediction of acute postoperative pain is good or simple enough to be considered useful in clinical practice.

Other ways of predicting acute postoperative pain

Boselli et al. proposed a new method for prediction of acute postoperative pain based on an analgesia/nociception index, calculated from heart rate variability during awakening (before extubation) from general anesthesia, claiming a sensitivity and specificity for prediction of acute postoperative pain of 86 % (ROC area 0.82). The test was designed to identify patients at risk of moderate to severe immediate postoperative pain. The timing of the test, just before awakening, makes it promising, enabling immediate treatment of susceptible patients. However, it does require specific equipment designed for this purpose (87).

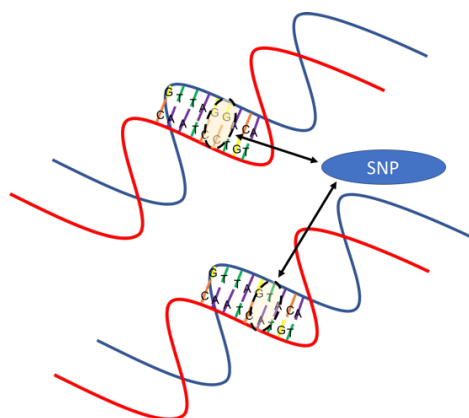
Orbach-Zinger published results indicating that pain associated with injection of local anesthetic (ILA) before spinal anesthesia for Caesarean section was associated with postoperative pain. Obvious differences in postoperative pain intensity were found between patients with mild or severe pain upon ILA. Patients with mild pain upon ILA on average graded their postoperative pain intensity at rest during the first 24 hours at 0.3 compared to 5.3 NRS in those with severe pain upon ILA (69). The statistical correlation of approximately 0.5 in this study is stronger than in most studies comparing experimental pain scores with postoperative pain levels.



Genetics and pain sensitivity

Genetic variation is believed to explain a large portion of pain variability. Studies in animal and human twins suggest that 30-60 % of this variance is explained by genetic factors. There are heritable conditions of reduced pain perception (HSAN I-V) and of increased pain like erythromelalgia, familial hemiplegic migraine and paroxysmal extreme pain. The variation of pain perception in the general population is a complex trait thought to depend on polygenic contributions not yet known (88).

How proteins are built up is determined by deoxyribonucleic acid (DNA) coding for specific protein structures. A gene is a specific DNA sequence on a particular portion of a chromosome encoding for synthesis of a specific protein. Alleles are different variants of the same gene. Replacing one nucleotide in a gene (point mutation) alters the gene and hence the corresponding coding for protein synthesis, which results in a slightly modified protein structure. Such variations can also result from DNA segments being eliminated, changing positions, or being inverted, in our genes. In some instances, a modified protein structure may lead to altered function of the protein and even cellular dysfunction.



A single nucleotide polymorphism (SNP) where a single base change has occurred in over 1 % of the population. In this case the nucleotide guanine has been exchanged for the nucleotide thymine.

A detectable variation of protein function is known as a phenotype, and polymorphism is when more than two phenotypes are present in a gene. Replacement of one nucleotide in less than 1 % of a population is called a mutation, and in more than 1 % a single nucleotide polymorphism (SNP). An allelic variation can be quiet and not at all affect the phenotype, but it can also cause individual differences in pharmacokinetic or pharmacodynamic properties of drugs, e.g. opioids (89).

In genetic association studies, the DNA in patients with a certain phenotype (e.g. pain condition) is compared with that in controls (wild-type). In candidate gene studies, already susceptible pain genes are compared between groups. In genome-wide association studies (GWAS), the entire genome is mapped and compared. The most recent method in this rapidly growing field of research is genome-wide arrays enabling objective comparison of SNPs across the entire genome.

Many have attempted the task of linking genetic variations to pain, and many different genes and SNPs are involved in pain sensitivity.

The most extensively investigated gene in this area is probably OPRM1, a gene coding for the μ -opioid receptor. The SNP A118G alters functional properties of the human μ -opioid receptor. The A118G polymorphism, where aspartate has been exchanged for asparagine, is found in 20 % of Caucasians. This allele confers a different nociceptive cerebro-cortical activation than the more common genotype (90). Subjects carrying one or two copies of the variant G allele have been found to have reduced analgesic response to alfentanil and morphine (91, 92) and also to need more morphine after surgery (93). Children with this polymorphism undergoing surgery are more likely to report high-intensity pain postoperatively (94). The G allele has also been associated with a lower electrical pain threshold and higher intra-operative need for fentanyl (95).

Catechol-O-methyl transferase (COMT), an enzyme involved in the metabolism of catecholamines, affects adrenergic and dopaminergic pathways involved in the pain response. It influences pain sensitivity by modulating neuronal transmission and is strongly associated with pain sensitivity (91). In several previous studies SNPs in this gene have been linked to pain or opioid responsiveness (96-99). Genetic variability at COMT modulates responses to opioids in acute postoperative pain (98). Individuals with low COMT activity have been shown to have higher pain sensitivity (100). However, the results have been questioned since other studies have reported no associations between certain SNPs in COMT and postoperative need for opioids (101).

The ABCB1 (previously called MDR1) is a gene encoding for a transporter part of the ABC superfamily of efflux transporters, which functions at capillary endothelial cells of the blood-brain barrier and blood-cerebrospinal fluid barrier. More than one hundred different SNPs are known in this gene. Many of them have been shown to influence pain sensitivity and have been proposed to be involved in opioid responsiveness after surgery (93, 102). The most investigated of the ABCB1 genetic polymorphisms, the non-synonymous exon 26 SNP (C3435T), has been found in 50-60 % of Caucasians (91).

There are several different SNPs in the above-mentioned genes that have been found to be involved in pain signaling. Regarding previous gene association studies, it should be emphasized that although many have been done in so called candidate pain genes, all of them seem to fail to replicate (75). In 2016, de Gregori et al. presented results from 200 patients going through abdominal surgery linking SNPs to opioid consumption and postoperative pain intensity. They analyzed eighteen SNPs in the OPRM1, COMT, UGT2B7 and ESR1 genes without finding any significant association with postoperative analgesia (103). Recently, a multicenter two-year study, including 90 SNPs and clinical factors in 500 cases and 500 controls, found no influence of genetic factors on development of PPSP (75).

In order to find associations, sought after by so many, some researchers advocate smaller studies with well-defined phenotypes to obtain more reliable and relevant results. Genotyping patients with clinical outcomes at either end of the scale may be more informative and cost efficient. Having a plan for a systematic research program at the start and a clear hypothesis is perhaps more important than testing for any polymorphism whose assay might be available (104).

Aims

Aims of this thesis were to investigate if the extent of postoperative pain

- can be predicted from assessments of pain induced by peripheral venous cannulation during routine preparation for surgery.
- can be predicted from assessments of pain associated with intravenous administration of propofol during induction of general anesthesia.
- can be predicted from electrical pain threshold levels.
- reflects genetic differences between patients reporting higher and lower levels of pain sensitivity and postoperative pain intensity.

Methods

The scientific papers included in this thesis were all based upon observational studies of prospective design at Halland's Hospital, a county hospital on the west coast of Sweden. All studies were approved by the Regional Human Research Ethics Review Board in Lund, Sweden. Study IV was also registered in Clinical Trials (<http://www.clinicaltrials.gov>).

Study participants

Paper I-II

One hundred-and-eighty patients scheduled for laparoscopic cholecystectomy were included in this prospective clinical study. The anesthetic protocol was highly standardized. Pain intensity associated with peripheral venous cannulation and administration of propofol, as well as levels of EPT, were recorded preoperatively. For evaluation of postoperative pain, we used assessments of pain intensity on a VAS, time to first rescue administration of opioid, and total dose of rescue opioid. Focus was on acute postoperative pain and the follow-up was 1.5 hours in the post anesthesia care unit (PACU).

Paper III

In this study, patients reporting the highest and lowest levels of pain intensity were chosen from the well-categorized cohort in study I. Individually reported levels of pre- and postoperative pain intensity were used for phenotype classification into a case (high-pain) group with VCP intensity > 2.0 and maximum postoperative pain intensity ≥ 7.0 VAS units, and a control (low-pain) group with ≤ 2.0 and < 4.0 VAS units, respectively.

Blood samples from 32 case and 25 control patients were used for DNA-extraction and genome-wide array to compare single nucleotide polymorphisms previously reported to be relevant for pain expression between the two study groups.

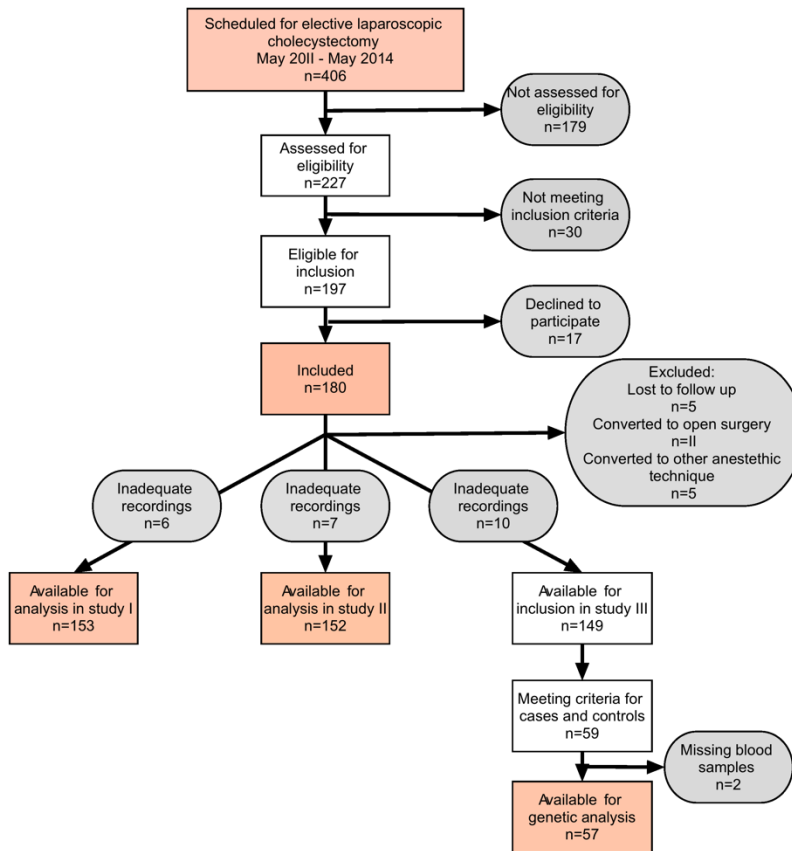


Figure 3 Inclusion flow chart study I-III
Description of inclusion of patients in the first three studies.

Paper IV

Six-hundred patients scheduled for elective surgery at Halland's Hospital Halmstad during three months in 2017, and prepared for surgery at the pre-operative unit, were included. All patients were asked to grade their VCP intensity on a VAS immediately after cannulation. The surgical procedures were categorized into four different groups,

based on presumed levels of postoperative pain, and the study patients were compared within these groups. The primary outcome measure was the reported maximum pain intensity in the PACU, and secondary outcome measures were the proportions of patients with moderate or severe pain, comparing patients with VCP intensity above or below 2.0 VAS units.

Preoperative tests of pain sensitivity



Venous cannulation-induced pain (I, IV)

The venous cannulation performed in study I was standardized to size of cannula and location as both factors are known to influence the pain associated with the procedure (51). We used the back of the hand and a venous cannula of inner diameter 1.1 mm. Immediately after the procedure patients were asked to grade their VCP on a horizontal VAS. The procedure was performed by several different nurses in our preoperative area and on a surgical ward at our hospital. In study IV the size of cannula and site for cannulation was optional for the nurse performing the procedure. The antecubital fossa was chosen for cannulation in most cases.



Pain associated with injection of propofol (I)

Before induction of anesthesia 3.0 ml of propofol (Propofol Lipuro®, Braun, Danderyd, Sweden) 10 mg/ml was injected over 5 seconds through the peripheral venous catheter on the back of the hand by the anesthetic nurse caring for the patient during surgery. The patient was then asked to grade their pain associated with the procedure on a horizontal VAS.



Electrical pain thresholds (II)

To determine electrical pain thresholds (EPT), we used a Painmatcher™ device (Cefar Medical AB, Lund, Sweden), delivering monophasic rectangular electrical pulses with a constant current of 15 mA and 10 Hz frequency on closure of a circuit between the thumb and index finger. To yield step-wisely increasing levels of pain intensity, the pulse duration increases gradually over time (from 4 to 396 μ s), thereby increasing the amount of energy delivered. When the patient releases the buttons of the device, a microprocessor-controlled arbitrary value (EPT score) between 1 and 99, reflecting the maximum energy delivered, is displayed. Each study patient was first carefully instructed to hold on, thereby letting the intensity of sensation increase until considered painful, and then let go. The EPT was determined three times in each study patient, and individual mean scores were calculated and recorded. The patients were blinded to their results.

Evaluation of postoperative pain (I, II, IV)

We have chosen to use the VAS for assessment of pain intensity as it offers the greatest opportunities for discrimination and to detect minute pain changes during analgesic administration. Our primary outcome parameter was pain intensity measured with a horizontal VAS. Secondary outcomes in study I-II, were time to first rescue opioid and total dose of rescue opioid within a certain time period. For our results to be considered clinically useful and relevant, all three measurements would have to lean the same way. The testing of VAS was performed by nurses trained in intensive care medicine, working at the PACU. In study IV secondary outcome was proportion of patients with moderate or severe pain after surgery. We chose to define slight pain as <VAS 4.0 and moderate pain as ≥ 4.0 .

Genetic testing (III)

The part of the chromosome coding for a gene is called the exome. The exome constitutes around 1 % of the genome and 180 000 exons. In genome-wide association studies the whole genome is mapped. In whole exome sequencing only the expressed genes are sequenced. Not sequencing the whole genome saves time and money and, more importantly, still allows for efficient identification of genetic bases responsible for inherited disorders in smaller cohorts. The large quantity of data obtained from these analyses require extensive knowledge and time for analysis and very large sample sizes. Exome sequencing may therefore be preferable in studies aiming towards identifying somatic mutations of clinical relevance. In complex disorders, like pain sensitivity, many genes are thought to be involved in disease risk and, especially then, limiting sequencing to the exome will be more efficient. The limitation of course being that potentially disease-causing variations in non-coding regions of the genome may be missed (105, 106).

With regard to the large amount of data and relatively small study groups we chose to focus on candidate pain genes in the bioinformatics analyses.

Whole exome array was performed at SciLife laboratories the SNP & SEQ Technology Platform, Department of Medical Sciences, Biomedical Centre, Uppsala University. Illuminas Omni Express Exome Array was used to analyze 964193 SNP markers from each blood sample. This array is set to sequence >240 000 functional exonic markers and >700 000 genome wide markers that provide coverage of common variants at >5% minor allele frequency (MAF). The results were then analyzed with GenomeStudio 2011.1 software (Illumina Inc., San Diego, California, USA). Quality control was performed using PLINK with 662 348 variants passing filters and quality control.

An experienced bioinformatician assisted in processing the results and a statistician with knowledge in genetic studies performed the statistical analysis of the genetic tests.

Statistical methods (I-IV)

Pain intensity levels are reported as median with interquartile range (IQR), considering the ordinal character of the VAS. Proportions are reported with 95% confidence interval (CI).

Continuous variables were compared between groups with the Mann-Whitney U-test (I, II, III, IV). The Pearson's χ^2 test was used to compare categorical variables, e. g. proportions (I, II, III, IV). The Kruskal-Wallis one-way analysis of variance was used to compare median values between different categories of surgical procedures (IV).

Correlations were analyzed with the Spearman's correlation test (I, II, IV). Time until first administration of opioid in the PACU was analyzed by Kaplan-Meier curves with log rank test (I, II). The ROC-curve was set up for determination of ideal thresholds (II). Predictive abilities were evaluated with logistic regression analysis and cross-tabulations set up to get sensitivity and specificity levels (I, II, IV). Associations between SNPs and pain sensitivity were investigated using linear regression (III). Levels of $P < 0.05$ were considered statistically significant (I, II, IV). The Bonferroni and permutation tests were used to adjust for multiple testing (III).

Results and comments

Peripheral venous cannulation – does it hurt?

Different levels of VCP intensity were recorded depending on the study setting. The median VCP intensity was found to be higher in patients scheduled for laparoscopic cholecystectomy (I) than for patients scheduled for various surgical procedures (IV). In study I the back of the hand was consistently cannulated with a venous catheter of inner diameter 1.1 mm in all patients. In study IV the size of catheter and site of cannulation was optional for the nurse performing the procedure and the antecubital fossa was chosen for cannulation in most cases. These differences most likely influenced the results obtained – median VCP intensity 2.5 versus 1.0 VAS units. This assumption is supported by the fact that patients cannulated on the back of the hand (IV) had similar median VCP intensity levels in studies I and IV, as also shown in Table 2.

Gender differences in pain experienced may also have influenced the results, as there is a trend towards higher reported pain intensity levels in women (Table 1).

Table 1. Venous cannulation-induced pain in different study settings.

Median (IQR) VCP intensity in all, female and male patients evaluated in different study settings and also reported by gender. Levels of statistical probability (*P*) represent differences between women and men. The last two rows contain previously unpublished data.

Procedure	Number of patients (women/men)	VCP all (VAS units)	VCP women (VAS units)	VCP men (VAS units)	P-value
Cholecystectomy in Halmstad (I)	153 (110/43)	2.5 (1.5-4.5)	3.0 (1.5-5.0)	2.0 (1.0-3.1)	0.6
Various surgical procedures in Halmstad (IV)	505 (330/175)	1.0 (0.2-2.5)	1.0 (0.4-3.0)	1.0 (0.0-2.0)	0.7
Blood donors in Halmstad	170 (68/102)	1.1 (0.4-2.6)	1.4 (0.7-3.6)	0.9 (0.2-1.9)	0.005
Various surgical procedures in Malmö	107 (24/83)	1.0 (0.3-1.9)			

Table 2. Venous cannulation-induced pain as influenced by site of cannulation and size of catheter.

Median (IQR) VCP intensity in 505 study patients cannulated on the back of the hand or in the antecubital fossa (IV), and with different (inner diameter 0.9 or 1.1 mm) catheter sizes (IV). Levels of statistical probability (P) represent differences between cannulation sites and catheter sizes.

	VCP (VAS units)	P-value
Site of cannulation		
Antecubital fossae (n = 387)	1.0 (0.0-2.1)	
Back of hand (n = 118)	2.0 (0.9-3.4)	<0.001
Size of catheter		
0.9 mm (n = 98)	1.0 (0.5-2.7)	
1.1 mm (n = 407)	1.0 (0.1-2.5)	0.3

Peripheral venous cannulation does hurt. It is a compulsory procedure during preparation for surgery often associated with pain intensity levels of 2.5-3 VAS- or NRS-units (48-50). We observed similar levels to be associated with cannulation on the back of the hand (I, IV), but in our follow-up study where most patients were cannulated in the antecubital fossa (IV), the median VCP was lower (1.0 VAS units), most likely since cannulation on the back of the hand is associated with more pain. We have also studied volunteers subjected to blood donation (unpublished data). We expected them to report less pain on venous cannulation in the antecubital fossa, since we assumed that individuals with higher pain sensitivity would be less likely to donate blood. However, considering that they were all cannulated in the antecubital fossa, their VCP intensity was the same as for surgical patients (Table 1), indicating again that location of cannulation is the factor that matters rather than setting. If our assumption of them experiencing less pain on venous cannulation is true the size of the cannula, being larger for blood donations, might have influenced the pain even though we have not found an influence of size of catheter in our other studies.

Can we use VCP measurements for prediction of acute postoperative pain?

Our results indicate that patients scoring cannulation-induced pain intensity > 2.0 VAS units have more pain after surgery, as they were given postoperative opioid earlier, more often, and in higher doses, and reported higher levels of postoperative pain intensity after elective laparoscopic cholecystectomy (I).

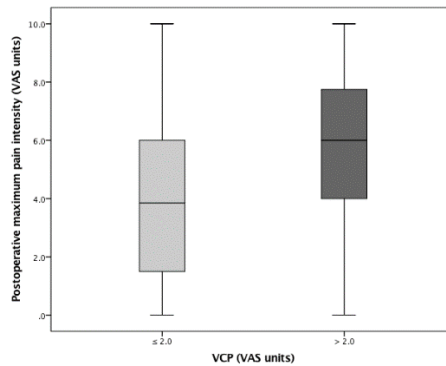


Figure 4. Postoperative pain intensity

Postoperative pain intensity in patients dichotomized according to pain intensity reported to be associated with peripheral venous cannulation (I). The boxes indicate IQR and the whiskers represent minimum and maximum values.

Significant correlations were found between VCP intensity and all outcome measures of postoperative pain (I). Patients scoring > 2.0 VAS units on venous cannulation were found to have 3.4 times higher risk of postoperative pain after laparoscopic cholecystectomy (Table 3).

Table 3.

Logistic regression analysis of the ability of venous cannulation-induced pain intensity levels (independent variable) to predict moderate or severe (≥ 4.0 VAS units) postoperative pain intensity (dependent variable), and the influence of potential confounders including gender, and age (I).

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Venous cannulation-induced pain intensity (VAS units)				
< 2.0	1.0		1.0	
≥ 2.0	3.4 (1.7-6.9)	< 0.001	3.4 (1.6-7.3)	< 0.005
Gender				
Men	1.0		1.0	
Women	1.8 (0.9-3.7)	0.1	1.1 (0.5-2.5)	0.8
Age (years)				
≤ 40	3.5 (1.3-9.2)	< 0.05	2.1 (0.8-6.0)	0.2
41-59	1.3 (0.6-2.9)		0.9 (0.4-2.1)	
≥ 60	1.0		1.0	

We performed a follow-up study in a large, very diverse group of patients going through different kinds of surgery with regional or general anesthesia provided (IV). In this cohort, we could confirm the results from study I showing that patients with VCP intensity ≥ 2.0 VAS units experienced higher levels of postoperative pain and more patients experienced moderate or severe postoperative pain (Table 4). The risk was higher (OR 1.7, $P = 0.005$) regardless of kind of surgery, type of anesthesia or other risk factors (Table 5).

Table 4.

Postoperative pain assessments after various surgical procedures in patients dichotomized for pain intensity associated with peripheral venous cannulation (IV). P-values indicate statistical difference between dichotomized patients.

	Venous cannulation-induced pain intensity		
	< 2.0 VAS units	≥ 2.0 VAS units	P-value
Maximum postoperative pain intensity (VAS units (median, IQR))	0.2 (0.0-4.0)	3.0 (0.0-5.0)	0.001
Proportion of patients with moderate to severe postoperative pain (n (%))	82 (26)	72 (38)	0.005
Total number of patients	315	190	

Table 5.

Logistic regression analysis of the ability of venous cannulation-induced pain intensity levels (independent variable) to predict moderate or severe (≥ 4.0 VAS units) postoperative pain intensity (dependent variable), and the influence of potential confounders including gender, and age (IV). Odds ratio is denoted by OR, and confidence interval by CI.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Venous cannulation-induced pain intensity (VAS units)				
< 2.0	1.0		1.0	
≥ 2.0	1.7 (1.2-2.6)	0.005	1.5 (1.0-2.3)	0.044
Gender				
Men	1.0		1.0	
Women	1.6 (1.0-2.4)	0.036	1.5 (1.0-2.2)	0.08
Age (years)				
≤ 40	1.5 (0.9-2.3)	0.002	0.5 (0.3-0.7)	0.006
41-59	2.4 (1.5-3.8)		0.6 (0.4-1.0)	
≥ 60	1.0		1.0	

On using VCP as a method to predict acute postoperative pain

Peripheral venous cannulation is somewhat painful but a necessary procedure when preparing for surgery. Hence, VCP measurements do not induce additional pain, take extra time or require specific equipment. Even though associations between pain intensity after surgery and results obtained with his technique may seem weak, its clinical potential is within grasp for any staff member, making it a useful tool in clinical bedside practice, preferably together with other recognized risk factors for postoperative pain. In a setting where factors like surgery type, premedication, and postoperative analgesic plan were standardized (I) the odds ratio for VCP to predict level of postoperative pain intensity was 3.4. In contrast, in a study setting comprising various kinds of premedication, surgeries, and postoperative analgesia (IV), the corresponding odds ratio was still found to be 1.7. This simple bedside test – easily and rapidly applicable before surgery under general or regional anesthesia – might hence be considered, preferably in combinations with other proposed techniques for pain prediction, to reflect individual needs for analgesia after surgery.

A few other research groups have also considered using pain induced by injection or cannulation for prediction of postoperative pain. Approximately when our initial results were published (I), Orbach-Zinger et al. published results indicating that pain associated with infiltration of local anesthetic before spinal anesthesia was associated with post-Caesarean pain (69), and previously, VCP intensity was associated with labor pain (55).

Can we use pain intensity associated with infusion of Propofol for prediction of acute postoperative pain?

Maximum postoperative pain intensity was found to be higher in patients experiencing pain over 2.0 VAS units to be associated with propofol infusion (I). However, as we did not find significant differences in the other parameters for postoperative pain evaluation (time to rescue opioid, total dose of rescue opioid) we consider this result less reliable. What we did find was, however, that patients who did not experience pain on either venous cannulation nor propofol infusion had low risk of postoperative pain ($P < 0.05$).

On using pain associated with infusion of propofol as a method to predict acute postoperative pain

Our results do not support the use of this method for prediction of postoperative pain. One could use the lack of pain as a factor that perhaps could indicate the patient being less pain sensitive.

Can we use electrical pain thresholds for prediction of acute postoperative pain?

In our second study (II), we investigated the use of electrical pain thresholds (EPT) for prediction of APOP. Significant correlations were found between EPT and maximum APOP intensity, time to first rescue opioid, and total dose of rescue opioid in the PACU. However, interaction test revealed significant influence of gender on the ability of EPT to predict APOP. Women with low EPT (< 15) had 4.5 times higher risk of moderate to severe APOP ($P = 0.003$). In men there was no predictive ability of this method (Table 6).

Table 6.

Logistic regression analysis of the ability of electrical pain thresholds (independent variable) to predict moderate or severe (≥ 4.0 VAS units) postoperative pain intensity (dependent variable), and the influence of potential confounders including gender, and age (II).

	Univariate analyses		Multivariate analyses	
	OR (95% CI)	<i>p</i> - value	OR (95% CI)	P - value
Women				
Electrical pain threshold				
< 15	5.2 (2.2-12.6)	0.000	4.5 (1.7-11.9)	0.003
≥ 15	1.0		1.0	
Age (years)				
≤ 40	1.8 (0.6-4.8)	0.027	0.9 (0.3-2.9)	0.2
41-59	5.0 (1.5-16.4)		2.4 (0.6-8.7)	
≥ 60	1.0		1.0	
Men				
Electrical pain threshold				
< 15	0.8 (0.2-2.9)	0.7	0.7 (0.2-2.7)	0.6
≥ 15	1.0		1.0	
Age (years)				
≤ 40	0.7 (0.2-7.4)	0.8	0.8 (0.2-3.3)	0.8
41-59	1.3 (0.2-7.4)		1.4 (0.2-9.1)	
≥ 60	1.0		1.0	

On using electrical pain thresholds to predict acute postoperative pain

Pain, being such a multifactorial symptom where both the experience itself and the measurements can be influenced by many different factors, is difficult to study and evaluate. Will we ever be able to measure pain sensitivity pre-operatively in an attempt to predict postoperative pain levels? A recent review on this topic concludes that there are no consistent associations between experimental pain testing and postoperative pain intensity despite extensive clinical research (34). Considering relatively weak associations found and conflicting results between different studies, it seems that most additional pre-operative tests, often complex and time-consuming, are of scientific interest only. Hope is set either to methods testing central pain mechanisms (temporal summation or CPM), or methods using supra-threshold stimuli (34). These methods are either quite complicated, requiring special equipment, time, and calm surroundings, and/or cause discomfort for the patient in an already stressful situation preparing for surgery. The review also mentions the question whether all these experimental tests are gender dependent (34). Most results reported to be strongly associated with postoperative pain have been obtained in

women (35, 67, 81, 84, 107-109) whereas non-significant results have been reported in men (33, 42, 44, 45, 79, 110-112).

Our study on prediction of APOP from EPT levels in patients of both genders (II), confirms an interaction of gender with significant associations in women only.

Although this result might be considered to trigger further research, it seems to us that weak statistical associations of EPT levels with pain intensity after surgery hardly justify its introduction in the already tight schedule preparing for surgery.

Can genetic variations explain differences in pain sensitivity?

We have also investigated the possible contribution of genetic SNPs to differences in pain sensitivity found in our cohort (III). None of 446 markers identified in thirteen candidate genes reached statistical significance on Bonferroni correction for multiple testing ($P = 0.00012$) or permutation testing. The minor alleles of the most important SNPs from the genetic analysis in the ABCB1 gene suggest a pain-sensitive phenotype both pre- and postoperatively (Table 7). We did find variations in already susceptible genes, where some minor alleles were linked to our pain-sensitive phenotype, but our results need to be confirmed in a larger cohort.

Table 7. Candidate gene studies

Genome-wide array displaying the 10 most important single nucleotide polymorphisms (SNP) within our candidate genes with their respective gene, location, nucleotides, minor allele frequencies, and statistics (III).

Gene	SNP	Minor/ Major allele	Minor allele frequency per phenotype		OR	P-value
			Cases (pain)	Controls (no pain)		
ABCB1	rs4728702	T/A	0.52	0.26	3.04	0.0060
ABCB1	rs1128503	A/G	0.5	0.26	2.85	0.0093
ABCB1	rs10276036	G/A	0.5	0.26	2.85	0.0093
ABCB1	rs868755	A/C	0.5	0.26	2.85	0.0093
ABCB1	rs11975994	G/A	0.5	0.26	2.85	0.0093
ABCB1	rs1202169	G/A	0.5	0.26	2.85	0.0093
ABCB1	rs1202168	A/G	0.5	0.26	2.85	0.0093
ABCB1	rs1202167	A/G	0.5	0.26	2.85	0.0093
COMT	rs9265	C/A	0.41	0.18	3.12	0.0094
COMT	rs2518824	C/A	0.41	0.18	3.12	0.0094

On using genetic tests to predict acute postoperative pain

With new methods for genetic analysis becoming more readily available, allowing fast, reliable, extensive and rather cheap mapping of genes, exomes or whole genome, huge amounts of data will be available to the researcher. This calls for large multicenter studies, including tens to hundreds of thousands of individuals, to attain enough statistical power. Between planning and realization of the study (III), the technical advances in the field were enormous. Large genome-wide arrays were suddenly both faster, easier and cheaper than other less extensive analyses. Although we knew that the large amount of data that would be available to us would make a significant result impossible, we decided to proceed with hypothesis-generating research. Despite the small cohort, we believe that we had a strong phenotype, considering that all patients were subjected to laparoscopic cholecystectomy under strictly defined peri-operative conditions. All patients were given the same pre-emptive analgesia, anesthetized with similar, short-acting drugs, and were given the same postoperative analgesia, including local analgesia administered by the surgeon. From this cohort of patients, we then selected patients ending up at the two extremes of the pain intensity scale, based on pre-operative tests and pain after surgery. To limit the risk of false positive (type I) error, we chose to limit our bioinformatics analysis to genes already claimed to be involved in pain signaling. Within these genes we decided to look at all polymorphisms, since previous studies have found presumably pain-modulating genes in which a large variation of SNPs may have a role. Although our results are primarily hypothesis-generating, we still find them interesting considering the clear phenotype.

Methodological issues

On why pain intensity of 2.0 VAS-units was chosen as cut-off...

In surgical patients cannulated on the back of the hand only, this level was found to be close to the median level of VCP intensity (I-III), and a ROC evaluating sensitivity and specificity also suggested this to be an appropriate cut-off level. However, this level was found to be high in surgical patients cannulated in the antecubital fossa (IV). Naturally, we have therefore considered lowering the cut-off level, but we strongly believe that being closer to the lower end of the VAS would make it more unreliable (113). We therefore suggest to keep the level at 2.0 VAS units for this clinical test.

On the decision to use resting pain scores...

In our studies, we have used scores of pain intensity at rest. Assessment of dynamic pain scores, pain during movement, breathing, and coughing, is thought to be more important for reducing risks of complications after surgery (17). High initial APOP intensity has frequently been linked to PPSP (24, 114, 115), where movement-evoked pain is considered to be a particularly strong predictor of PPSP (24). One could argue that a dynamic indicator of pain might have been a better endpoint for our studies than using a static one, i.e. e. pain at rest. As pain scores during movement tend to be higher (116), they might also be more useful to confirm therapeutic effects, considering that it is easier to induce a large decrease in measured pain with analgesic drug if the initial level of pain intensity is higher (17). However, opioids, being our first line of treatment for APOP, seem to have better effect on pain at rest than on pain during movement (116). Dynamic assessments of pain are certainly both interesting and useful, but during our brief follow-up periods (I-IV) we considered frequent assessments of pain intensity mandatory to actually catch the highest reported individual levels of APOP (called maximum pain intensity). All APOP measurements were done by PACU-nurses, and to enable appropriate data collection, we preferred frequent assessments at rest to less frequent assessments at both rest and coughing.

On why patients with recurrent habitual pain were not included...

We considered lack of recurrent pre-operative pain a prerequisite for study inclusion (I-III), since we believed habitual pain to potentially influence the intensity of APOP, but few patients were considered non-eligible for this reason. Questions may be raised against not including study patients for this reason, considering that pre-operative pain is a common clinical indication for cholecystectomy. However, even if pain would be the primary reason for healthcare contact and diagnosis, usually these patients experience pain when they have their cholecystitis, and are then scheduled for surgery approximately three months later, when the inflammation has settled and they no longer suffer from pain. In support of this, according to a recent study (122), patients scheduled for elective laparoscopic cholecystectomy had no pain on inclusion (median 0 VAS units). In the follow-up study (IV) we included patients regardless of pre-operative pain and still found an association between VCP and APOP.

On postoperative pain intensity as outcome measure...

Pain is a complex symptom to measure and treat. With that said, it is also difficult to define an outcome measure that truly reflects the pain experienced. We chose to define a primary outcome measure based on determination of the intensity of APOP, but also to use an integrative assessment of pain scores, where pain intensity, dose of rescue analgesic, and time to its administration should be uniform for results to be considered relevant and significant. A similar method has previously been proposed, even though no uniform way to define and measure postoperative pain has been widely adapted (117).

The fact that we involved trained ICU nurses for pain measurements and parallel recording of opioid doses given at defined minimal pain intensity levels (>4.0 VAS units) further reinforces our results. Different methods of combining information on pain intensity and analgesic use into one integrative score have been proposed in order to provide a combined single primary outcome measure (118, 119). Letting PCA doses reflect individual pain intensity levels and opioid use is more tricky, since patients titrate their administration of analgesic drugs at different levels of pain intensity (118). Yet another important factor to standardize is the duration of follow-up of APOP. Added daily levels of APOP intensity on days 1-7 have been reported to better predict PPSP than the maximum intensity of APOP (114). Although individual follow-up of APOP comprised few hours in each study patient (I-IV), it still reflects supervision in the PACU allowing pain intensity and analgesic use to be reliably assessed and consistently recorded by trained ICU nurses.

On non-parametric statistical methods...

Some argue that scoring pain intensity along a ten-centimeter linear scale enables accurate continuous measurements and hence the use of parametric tests (120). However, many (like us) consider the VAS to be ordinal, meaning that e. g. pain intensity scored at 4.0 does not necessarily mean twice of the intensity corresponding to 2.0 VAS units (121). It has also been shown that the magnitude of a clinically relevant change in pain intensity depends on where on the scale the individual patient starts out. Since clinically significant changes in pain intensity differ along the VAS (21), the appropriate descriptive and analytical statistics to be used are non-parametric (117, 118, 121), and accordingly we have used statistical tests for non-parametric data.

On the use of psychometric evaluations...

In a meta-analysis on psychological evaluations and postoperative pain only 55 % of the included studies reported associations between pre-operative anxiety or pain catastrophizing and PPSP. The pooled OR based on 15 studies ranged from 1.55 to 2.10 (77), meaning that the risk increase in those studies actually showing a statistically significant association is lower than that shown with our proposed VCP-test (I, II, IV). In a recent study, where PCS was considered to significantly predict dynamic APOP intensity after 24 hours, the AUC was only 0.65 (63). In contrast, other studies have shown strong correlations between PCS and APOP intensity as well as opioid use (71). Adding psychometric data might have improved our understanding of mechanisms behind the predictive ability of the VCP-test (I, IV), and perhaps also provided information regarding mental health and development of PPSP. Also, it might have rendered us with a possibility to produce a risk index including VCP-measurements, but this remains to be investigated.

Conclusions

From results obtained in this thesis we conclude that

the assessed pain intensity induced by peripheral venous cannulation is useful for prediction of postoperative pain, since patients with cannulation-induced pain at or above 2.0 VAS units had considerably higher risk of moderate to severe acute pain after surgery (I, IV).

painful pre-operative routine procedures can be used to predict postoperative pain, considering that low pain intensity associated with both peripheral venous cannulation and intravenous administration of propofol indicate lower risk of acute moderate to severe postoperative pain (I, IV).

electrical pain threshold levels determined in the pre-operative period can be used to predict postoperative pain in women but not in men, considering that thresholds correlated weakly with pain intensity after laparoscopic surgery in women, but not at all in men (II).

single nucleotide polymorphisms in the genes ABCB1 and COMT possibly contribute genetically to individual pain sensitivity, considering that minor-allele single nucleotide polymorphisms in the genes were found to be more common in patients with higher pain sensitivity and intensity levels (III).

Future perspectives

Even though one can argue that predicting acute postoperative pain in an attempt to improve treatment is important, the ability to predict who will end up with persisting pain would be the ultimate goal. With this purpose, we are in the process of performing a follow-up of the patients from study I and II at five years after surgery. Whether or not VCP measurements can be used to direct individual treatment, and thereby reduce levels of postoperative pain, remains to be investigated.

One might also consider trying to introduce VCP as part of a predictive index score, which might render us with a simple and readily available bedside method with better predictive abilities.

The most interesting single nucleotide polymorphisms from paper III, being hypothesis generating, deserves to be investigated in a new cohort.

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Prediction of postoperative pain from assessment of pain induced by venous cannulation and propofol infusion

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Conflicts of interest

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Background: Postoperative pain may lead to delayed mobilization, persisting pain, and psychosocial distress. There are no simple and reliable techniques for prediction of postoperative pain. This study was designed to evaluate if pain induced by venous cannulation or propofol injection can be used to predict postoperative pain.

Methods: This prospective study included 180 patients scheduled for laparoscopic cholecystectomy. Pain intensity associated with peripheral venous cannulation and administration of propofol preoperatively and pain intensity, and use of opioid postoperatively was recorded.

Results: Patients scoring cannulation-induced pain intensity > 2.0 VAS units were given postoperative opioid more often (65% vs. 36%; $P < 0.001$), earlier (12 min vs. 90 min; $P < 0.001$), and in higher doses (4.8 mg vs. 0 mg; $P < 0.001$), and also reported higher levels of postoperative pain intensity (5.8 vs. 2.9 VAS units; $P < 0.001$). There were also significant ($P < 0.01$) correlations with postoperative pain intensity ($r_s = 0.24$), time to opioid administration ($r_s = -0.26$), and total dose of opioid ($r_s = 0.25$). Propofol-induced pain intensity correlated significantly ($P < 0.05$) with postoperative pain intensity ($r_s = 0.19$).

Conclusion: Pain intensity associated with venous cannulation and propofol infusion can easily be evaluated at bedside before surgery without specific equipment or training. Patients scoring > 2.0 VAS units on venous cannulation were found to have 3.4 times higher risk of postoperative pain after laparoscopic cholecystectomy. Low pain intensity associated with venous cannulation and propofol infusion indicate lower risk of postoperative pain.

Editorial comment: what this article tells us

These findings demonstrated that patients scoring > 2.0 VAS units during venous cannulation had 3.4 times higher risk of postoperative pain after laparoscopic cholecystectomy. As this measure can easily be evaluated before surgery, it may serve as a simple predictor of severe acute pain after a surgical procedure.

Acute postoperative pain increases the risks of delayed mobilization, venous thromboembolism, systemic infection, and opioid-associated adverse events^{1,2}. Long-term consequences of acute postoperative pain include persisting pain in 10–50%³, and physical disability and psychosocial distress in 5%^{4,5}. In 2005, Bisgaard et al. reported that 11% of patients met clinical criteria for chronic pain at 1-year follow-up after laparoscopic cholecystectomy⁶. Pre-existing pain and high-intensity postoperative pain are both known predictors for development of persisting pain after surgery^{6–12}. Preoperative identification of patients at risk of developing intense postoperative pain is therefore most desirable to further optimize individual pain treatment.

Patient age and gender, as well as psychological factors such as expectations of a painless postoperative course, have been shown to influence postoperative outcome with regard to pain^{13,14}. Even better predictive strength has been attributed to experimental induction of pain for evaluation of pain sensitivity. Postoperative pain intensity^{15–25} has been reported to correlate with different modalities of preoperative quantitative sensory testing, including assessment of pain thresholds to electrical^{17,24,26}, cold^{15,16,18,21,22}, heat^{16,18,22}, and pressure¹⁹ stimulation. Those results have not been overwhelming, and none of these tests have led to routine use for prediction of postoperative pain, partly because additional measures are required outside preoperative routine procedures. Thus, there is currently no simple and reliable technique for individual bedside prediction of postoperative pain in clinical anesthetic practice.

The aim of this study was to test if pain intensity associated with two preoperative routine procedures – peripheral venous cannulation and intravenous infusion of propofol²⁷ – could be used to predict the occurrence of postoperative pain.

Methods

In this prospective clinical observational study, we included adult patients scheduled for laparoscopic cholecystectomy at two county hospitals with identical anesthesiological routine procedures, in cities with similar socioeconomic

demography, on the west coast of Sweden. Elective laparoscopic cholecystectomy is carried out mainly by resident surgeons at the hospital in Halmstad, and by more experienced specialist surgeons at the (slightly smaller) hospital in Kungälv. Patients in Halmstad typically stay overnight while these are usually outpatient procedures in Kungälv. Site visits and follow-ups were made regularly to ascertain identical clinical procedures.

Primary outcome measures were the relationships between preoperative bedside visual analog scale (VAS) assessments²⁸ of pain intensity ≤ 2 or > 2 VAS units, associated with venous cannulation and propofol infusion, and postoperative pain estimates. The primary endpoint was postoperative pain intensity. Secondary endpoints were time to first rescue dose of opioid and total dose of opioid.

The regional Ethical Review Board at Lund University, Lund, Sweden, approved the study on 16th September 2010 (Dnr: 2010/391).

Study population

The study patients were informed about the study in writing a few weeks before the operation and orally upon arrival at the hospital in the morning on the day of surgery.

Patients considered eligible were consecutively assessed for inclusion criteria (scheduled for elective laparoscopic cholecystectomy, aged 18–80 years, ASA (American Society of Anesthesiology) classification I–II, ability to understand instructions, no recurrent preoperative pain, no contraindication to the premedication). Recurring preoperative pain was defined as daily pain, pain associated with regular use of analgesic drugs, or any other chronic pain condition.

Patients meeting inclusion criteria were included in the morning of surgery, after oral and written informed consents, assigned inclusion numbers in the order in which they had been included, and informed on how to use a horizontal VAS slide ruler for assessment of pain intensity. The study investigators were responsible for inclusion and information of the patients on arrival at the hospital. Patients were considered for inclusion when investigators were available at their workplace.

Venous cannulation test

A superficial vein on the back of the hand was cannulated with a standard-size (1.1 mm inner diameter) peripheral venous catheter (Venflon™, Becton-Dickinson, Helsingborg, Sweden) by nurses in the preoperative or surgical areas. These nurses were aware of whether the patient was to take part in the study but different from nurses involved in follow-up.

The patients were asked to estimate, on a horizontal VAS ruler, their maximum pain intensity associated with this procedure, recorded to one decimal point (0.0–10.0), and were then dichotomized according to VAS levels ≤ 2.0 or > 2.0 .

Premedication

Soon after venous cannulation and before infusion of propofol, the patients were given oral etoricoxib 120 mg, paracetamol 1500 mg (1000 mg if < 50 kg of body weight), slow-release oxycodone 10 mg (5 mg if > 65 years of age or < 50 kg of body weight), and ondansetron 8 mg. Betamethasone 4 mg was given intravenously (i.v.) before induction, immediately after the propofol infusion test.

Propofol infusion test

In the operating room, a small i.v. dose (30 mg) of propofol (Propofol Lipuro®, Braun, Danderyd, Sweden) was injected over 5-s through the catheter on the back of the hand. The patients were asked to estimate, on a horizontal VAS ruler, the maximum pain intensity associated with this procedure, recorded to one decimal point (0.0–10.0), and were then dichotomized according to VAS levels ≤ 2.0 or > 2.0 .

Perioperative procedures

Anesthesia was induced with i.v. propofol and remifentanyl, and maintained with propofol and remifentanyl by target controlled infusion (Alaris® CC Plus Syringe Pump using the Marsh model provided by CareFusion, Sollentuna, Sweden), based on standard algorithms for estimation of appropriate plasma concentrations according to age, weight, and gender. The target plasma concentration of propofol was set at

3–4 µg/ml, and target plasma concentrations of 3–8 ng/ml of remifentanyl were then used to adjust the level of anesthesia according to individual needs. Rocuronium (0.5 mg/kg i.v.) was given before endotracheal intubation. Before the start of surgery, 0.4 ml/kg of ropivacain 7.5 mg/ml was injected around the entrance holes and over the liver bed.

Laparoscopic cholecystectomy was performed using one 10-mm and two 5-mm trocars. The gallbladder was retracted via the sub-umbilical 10-mm port site. Intra-abdominal pressure was maintained at 10–12 mmHg with carbon dioxide and this gas was evacuated at the end of surgery. The fascia in the wider port was closed with resorbable suture, and the skin at all port sites with non-resorbable suture or metal clips.

Approximately 30 min before turning off the infusion of remifentanyl, the patient was given i.v. morphine 0.2 mg/kg (up to a total dose of 15 mg).

Postoperative pain assessment and rescue treatment

Postoperative pain intensity at rest, estimated to one decimal point (0.0–10.0 VAS units), was assessed from arrival in the post-anesthesia care unit and at 10, 20, 30, 60, and 90 min in awake patients. In patients reporting values ≥ 4.0 VAS units, defined i.v. doses of opioid (morphine 2.5 mg) were given at 5-min intervals until levels below 4.0 VAS units were reported. Measures of postoperative pain were evaluated by pain intensity ratings, time from extubation to first dose of opioid, and total dose of opioid within 90 min.

Statistical analysis

A total number of 151 patients was calculated to be required to confirm, with 95% statistical probability and 80% power, a difference (with a confidence interval (CI) of at least 8%) in proportion of patients with maximum postoperative pain intensity levels < 4.0 VAS units between those reporting venous cannulation and propofol infusion to be associated with levels \leq or > 2.0 VAS units, respectively. Statistically significant associations of postoperative pain intensity or opioid use with the intensity of

experimental pain (induced by heat or cold) has been statistically confirmed in this number²⁹, and also in one-third of this number^{16,20,22}, in surgical patients subjected to obstetric, gynecological or abdominal procedures. However, 180 patients were primarily included to allow for up to 20% dropouts.

Results are reported as median with interquartile range (IQR) except for proportions, where 95% CI is reported.

Statistical tests for nonparametric data were used. Continuous variables were compared between groups with the Mann–Whitney *U*-test. The Pearson's chi-square test was used to compare categorical variables. Correlations between variables were analyzed with the Spearman's rho correlation test (r_s). Kaplan–Meier curves were used to evaluate time to rescue opioid with the log rank test, and the Mann–Whitney *U*-test was used for group comparisons. A cross-tabulation was set up for calculation of predictive levels of moderate and severe pain. Logistic regression analysis was used to adjust predictive abilities of cannulation-induced pain intensity for age and gender.

The IBM SPSS version 20.0 and 23.0 software packages (IBM Inc., Armonk, NY, USA) were used for statistical analyses.

Values of $P < 0.05$ were considered statistically significant.

Results

In total, 406 patients were scheduled for elective laparoscopic cholecystectomy at the two study sites, and 227 of them were assessed for eligibility. 180 patients were primarily included (118 patients in Halmstad between May 2011 and May 2014 and 35 patients in Kungsbäck between October 2013 and May 2014). Some individual data were missing in four patients, and information obtained in the remaining 149 patients was analyzed statistically (Fig. 1).

Study site

There were no differences between the two study sites in patient age [48 (IQR 38–66) vs. 48 (37–55) years], weight [79 (68–90) vs. 78 (72–91) kg], gender [Halmstad 74 (95% CI 66–82)% women vs. Kungsbäck 69 (54–84)% women],

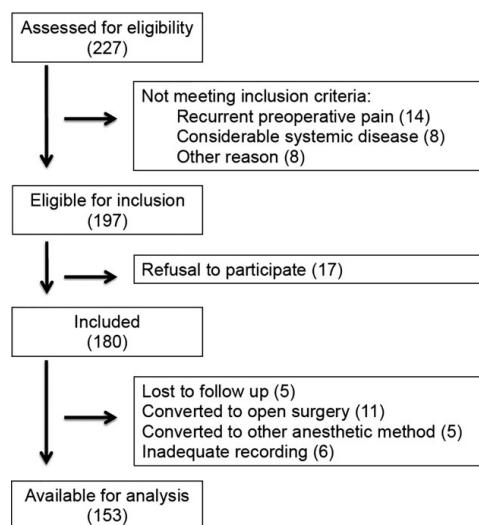


Fig. 1. Inclusion flow chart. Reasons for not meeting inclusion criteria were: the patient was prepared by another nurse (3), unable to understand instructions (1), age (1), and language issues (3). Inadequate recordings not making analysis possible were missed documentation of a considerable part of the follow up.

or dose of remifentanyl [1.76 (1.44–2.24) mg vs. 1.70 (1.37–2.04) mg], but the duration of surgery was significantly ($P < 0.001$) longer in Halmstad [110 (95–147) min] than in Kungsbäck [83 (72–107) min]. There were no significant differences in pain intensity associated with venous cannulation [2.8 (1.5–5.0) vs. 2.0 (1.0–3.2) VAS units] or propofol infusion [1.0 (0.0–3.8) vs. 0.0 (0.0–3.5) VAS units], or in maximum postoperative pain intensity [5.0 (2.5–7.2) vs. 4.0 (1.4–6.8) VAS units], time to first rescue dose of opioid [30 (3–90) vs. 60 (11–90) min], and total dose of morphine within 90 min [2.5 (0.0–5.8) vs. 1.3 (0.0–5.0) mg], between study patients managed in Halmstad or Kungsbäck, respectively.

Gender

There were significant ($P < 0.05$) differences between men and women in proportion given opioid within 90 min [men 48 (95% CI 33–63) vs. women 67 (57–75)%] and in time to first

dose of opioid [90 (IQR 5–90) vs. 16 (2–90) min], but not in total dose of opioid administered. According to univariate logistic regression analysis, gender was not a significant risk factor of postoperative pain and did not influence the predictive ability of venous cannulation in the multivariate analysis adjusted for age and gender (Table 1).

Age

There were significant correlations between patient age and maximum postoperative pain intensity ($r_s = -0.25$; $P < 0.01$), use of opioid ($r_s = -0.26$; $P < 0.01$), time to first dose of opioid ($r_s = 0.28$; $P < 0.001$), and total dose of opioid administered ($r_s = -0.26$; $P < 0.001$) within 90 min.

Age was found to be a significant ($P < 0.05$) risk factor for postoperative pain in the univariate logistic regression analysis, but did not influence the predictive ability of venous cannulation in the multivariate logistic regression model adjusted for age and gender (Table 1).

Venous cannulation test

The cut-off point of categorization according to pain intensity associated with venous cannula-

tion (2.0 VAS units) was found to correspond closely to the median level of pain intensity reported (2.5 (IQR 1.5–4.5) VAS units). Maximum postoperative pain intensity (Fig. 2A), and total dose of opioid, were both significantly ($P < 0.001$) higher in patients reporting cannulation-induced pain intensity > 2.0 VAS units (Table 2).

The proportion of patients given no postoperative opioid within 90 min was significantly ($P < 0.001$) higher among those reporting cannulation-induced pain intensity levels of ≤ 2.0 VAS units than in those with levels > 2.0 (Table 2, Fig. 2B). Patients scoring > 2.0 VAS units were also given postoperative opioid

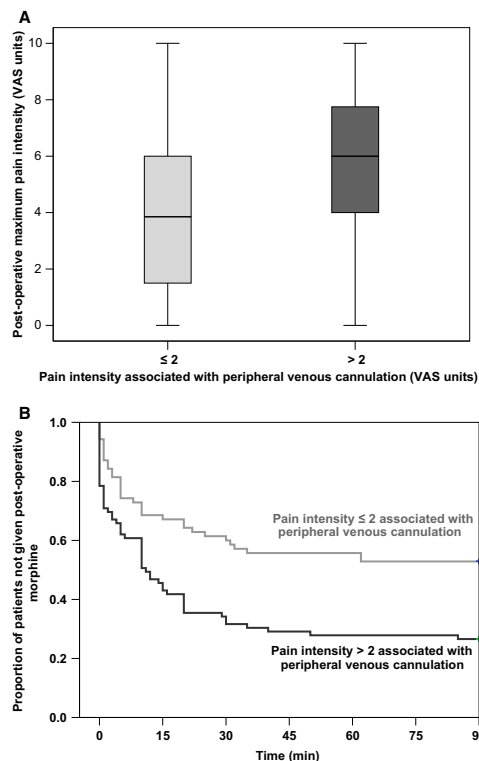


Fig. 2. Postoperative pain intensity (A) and opioid consumption (B) in patients dichotomized according to pain intensity reported to be associated with peripheral venous cannulation. The whiskers represent minimum and maximum values.

Table 1 Logistic regression analysis for the ability of preoperative pain scores (≤ 2) associated with venous cannulation to predict postoperative pain intensity ≥ 4 , adjusted for age and gender.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Pain intensity at venous cannulation (VAS units)				
≤ 2	1.0 (ref)	< 0.001	1.0 (ref)	< 0.005
> 2	3.4 (1.7–6.9)		3.4 (1.6–7.3)	
Age (years)				
≤ 40	3.5 (1.3–9.2)	< 0.05	2.1 (0.8–6.0)	0.2
41–59	1.3 (0.6–2.9)		0.9 (0.4–2.1)	
≥ 60	1.0 (ref)		1.0 (ref)	
Gender				
Men	1.0 (ref)	0.1	1.1 (ref)	0.8
Women	1.8 (0.9–3.7)		1.1 (0.5–2.5)	

Table 2 Demographics and postoperative pain assessments in patients dichotomized for pain intensity associated with peripheral venous cannulation.

	Patients reporting pain intensity (VAS units) associated with peripheral venous cannulation at		P-value
	≤ 2	> 2	
Female gender % (95% CI)	61 (49–73)	81 (70–89)	< 0.01
Age years [median (25–75% IQR)]	54 (45–68)	46 (31–54)	< 0.001
Body weight kg [median (25–75% IQR)]	83 (73–92)	75 (69–90)	0.12
Duration of surgery min [median (25–75% IQR)]	110 (82–158)	104 (84–122)	0.09
Hospital Halmstad;Kungsbacka	51;19	63;16	NS
Postoperative maximum pain intensity VAS units [median (25–75% IQR)]	2.9 (1.2–6.0)	5.8 (4.0–8.0)	< 0.001
Postoperative time to first administration of morphine min [median (25–75% IQR)]	90 (10–90)	12 (1–90)	< 0.001
Total administration of postoperative morphine within 90 min mg [median (25–75% IQR)]	0 (0–2.5)	4.8 (2.5–7.5)	< 0.001
Patients not given postoperative morphine within 90 min % (95% CI)	64 (52–75)	35 (25–47)	< 0.001
Total number of patients	70	79	

VAS, visual analog scale; CI, confidence interval; P, statistical probability.

significantly ($P < 0.001$) earlier than those scoring ≤ 2.0 (Table 2, Fig. 2B).

There were significant ($P < 0.01$) correlations between pain intensity associated with venous cannulation and maximum postoperative pain intensity ($r_s = 0.24$), time to first rescue dose of opioid ($r_s = -0.26$), and total dose of opioid ($r_s = 0.25$) within 90 min.

A cross-tabulation model was set up to identify patients with slight postoperative pain (< 4.0 VAS units) within 90 min (Table 3). Of patients with levels of cannulation-induced pain intensity ≤ 2.0 VAS units, 50% reported slight (< 4.0 VAS units) ($P < 0.001$), and 21% severe (≥ 7.0 VAS units; $P < 0.05$) postoperative pain. Among patients rating pain intensity on venous cannulation > 2.0 , 23% reported slight ($P < 0.001$), and 38% severe ($P < 0.05$) postoperative pain. The positive predictive value of this test was 77% (95% CI 66–86).

Patients reporting pain intensity levels > 2.0 VAS units to be associated with venous cannulation had 3.4 times higher risk of postoperative

Table 3 Cross-tabulation for a prediction model for postoperative pain depending on pain associated with peripheral venous cannulation.

	Patients reporting pain intensity (VAS units) associated with peripheral venous cannulation		Total numbers of patients
	≤ 2	> 2	
Patients reporting maximum postoperative pain intensity (VAS units)			
< 4	35	18	53
≥ 4	35	61	96
Total numbers of patients	70	79	149

Comparison of the number of patients experiencing pain exceeding VAS 4 in the post-anesthesia care unit depending on their pain associated with venous cannulation before the operation ($P < 0.001$). VAS, visual analog scale.

pain after adjustments for gender and age (Table 1).

Propofol infusion test

The cut-off point of categorization according to pain intensity associated with propofol infusion (2.0 VAS units) was set at the same level as for pain induced by venous cannulation. Maximum postoperative pain intensity was significantly ($P < 0.05$) higher in patients reporting propofol-induced levels of pain intensity > 2.0 VAS units (Fig. 3A), whereas there were no differences in use, time to first dose, or total dose, of opioid (Fig. 3B).

There was a significant ($P < 0.05$) correlation between propofol-induced and maximum postoperative pain intensity ($r_s = 0.19$). No signifi-

cant correlations were found with use, time to first dose, or total dose, of opioid within 90 min.

Individually reported levels of pain intensity ≤ 2.0 VAS units associated with both venous cannulation and propofol infusion were significantly ($P < 0.05$) associated with lower estimates of pain (maximum intensity, time to opioid, and total dose of opioid) in the early postoperative period (Table 4). Fewer ($P < 0.005$) of these patients [44 (95% CI 30–59) vs. 68 (59–76)%] were given opioid within 90 min in the postoperative period.

Discussion

To our knowledge, this is the first study to show that preoperative bedside assessment of pain intensity associated with common procedures in anesthetic routine practice can be used to predict postoperative pain.

Peripheral venous cannulation, a mandatory procedure in any patient scheduled for surgery, is more or less painful in agreement with the median level of pain intensity (2.5 VAS units) reported here. We have shown that patients rating pain associated with venous cannulation > 2.0 VAS units have more intense postoperative pain, as also reflected in earlier and more administration of opioid. A recognized aim in clinical anesthetic practice is to keep postoperative pain intensity at tolerable levels (i.e. below 4.0 VAS units). By primarily dichotomizing patients as described above, we were able to identify approximately three of four patients in need of particular postoperative attention and analgesic management, where patients scoring > 2.0 VAS units on venous cannulation were found to have 3.4 times higher risk of postoperative pain.

We consider these tests to be clinically relevant to bedside practice. No specific resources, time, or equipment are required.

Postoperative pain has been reported to be moderate in 30%, and severe in 11%, of surgical patients³⁰, and despite global efforts there has been no recent improvement in postoperative pain relief³⁰. Known risk factors are female gender, lower age, preoperative pain, surgical nerve damage, and certain more extensive surgical procedures^{4,12,31–33}. As acute pain – the most frequent complaint after laparoscopic cholecystectomy³⁴ – is significantly associated with

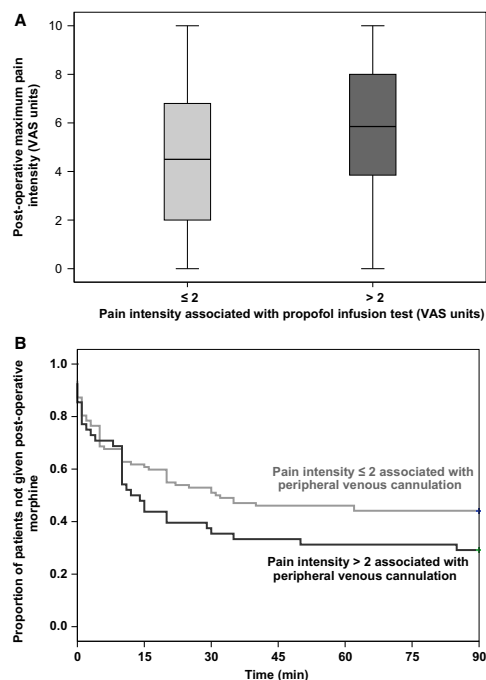


Fig. 3. Postoperative pain intensity (A) and opioid consumption (B) in patients dichotomized according to pain intensity reported to be associated with peripheral intravenous infusion of propofol. The whiskers represent minimum and maximum values.

Table 4 Postoperative pain according to preoperative pain assessments after venous cannulation and propofol infusion test.

	Pain intensity (VAS units) associated with peripheral venous cannulation	
	≤ 2	> 2
Pain intensity (VAS units) associated with intravenous infusion of propofol		
≤ 2		
Number of patients	57	42
Maximum postoperative pain intensity (VAS units)	3.2 (1.3–5.0)	5.9 (3.2–7.8)
Time to first postoperative administration of morphine (min)	90 (18–90)	10 (0–90)
Total postoperative dose of morphine within 90 min (mg)	0.0 (0.0–2.5)	5.0 (0.0–9.4)
> 2		
Number of patients	13	34
Maximum postoperative pain intensity (VAS units)	6.0 (1.6–8.3)	5.9 (4.0–7.9)
Time to first postoperative administration of morphine (min)	20 (2–90)	13 (2–89)
Total postoperative dose of morphine within 90 min (mg)	2.5 (0.0–9.5)	2.5 (0.3–5.0)

Postoperative pain intensity, opioid consumption, and time to first rescue opioid in study patients reporting pain \leq 2 VAS units, respectively, associated with intravenous infusion of propofol (in rows) and peripheral venous cannulation (in columns). Data are presented as median (25–75% interquartile range). VAS, visual analog scale.

development of chronic pain⁶, managing these patients more appropriately with respect to pain in the early postoperative period might have potential long-term benefits. Methods for rapid bedside prediction of postoperative pain are most desirable.

Cannulation-induced pain intensity scores were found to be clinically useful, although the procedures of evaluation involved many nurses. Although not our original intention, for practical reasons we had to use different nurses in different hospital areas for venous catheterization and corresponding pain evaluation. Nevertheless, we consider this approach to reflect more authentic clinical conditions, and hence the results to be more generalizable. It therefore seems that pain associated with peripheral venous cannulation can be readily scored independent of location or provider while preparing for elective surgery.

Concerning the propofol infusion test, it cannot be excluded that propofol-induced local pain intensity was to some extent alleviated by the strategy of premedication³⁵. This might have rendered prediction of earlier or higher use of opioid more difficult, whereas maximum postoperative pain intensity was still predictable. As various aspects on postoperative pain are interconnected, those circumstances still seem to make this test somewhat less reliable. On the other hand, there was a trend toward similar

results as for the cannulation test regarding associations with use, time to first dose, and total dose, of rescue opioid. Moreover, patients with pain intensity levels \leq 2.0 VAS units in both tests, were found to be those with the lowest levels of postoperative pain and administration of opioid.

Even though the study was carried out at two hospitals, there was no significant difference in outcome data between those study sites. Non-significant trends toward higher postoperative pain intensity scores, shorter time to rescue analgesia, and higher doses of opioid in Halmstad might have been due to a higher proportion of surgeons in training compared with in Kungsbäck, as also reflected in longer duration of surgery. Nevertheless, this difference did not result in statistically different levels of postoperative pain.

In conformity with previous findings^{13,14}, we found a negative correlation between age and estimates of postoperative pain, where younger patients reported more pain. Our finding that women have more postoperative pain and require more opioid than men is also in agreement with findings by others^{13,14,36}. Accordingly, younger patients and females also reported higher levels of pain intensity to be associated with venous cannulation. Nevertheless, we have shown individual scoring of can-

nulation-induced pain intensity to be another useful predictor of postoperative pain, not influenced by age or gender according to multivariate logistic regression analysis.

Persistent postoperative pain may result from already susceptible pain pathways³⁷, and as preoperative pain is a significant predictor of severe postoperative pain^{4,14}, we chose not to include patients with considerable preoperative pain. Acute pain has also been proposed to be an important predictor of chronic pain^{4,37}, presumably by upgrading mechanisms of pain signaling and perception³⁷. One might therefore assume that individualizing the analgesic regime to reduce the number of patients with unacceptable levels of acute pain early after surgery might reduce the incidence of persistent postoperative pain. Our finding that three in five surgical patients reported moderate levels of postoperative pain (≥ 4.0 VAS units) is far from acceptable.

Several recent studies have focused on prediction of acute postoperative pain. Responses to quantitative sensory testing induced by heat or pressure have been reported to correlate fairly well with the intensity of postoperative pain^{16,17,22,29}, although some studies contradict those findings^{25,26}. In a review from 2010, Werner et al. conclude that preoperative pain tests may predict up to 54% of the variance in postoperative pain experience, and that this predictive strength is higher than previously reported for demographic and psychological factors³⁸. The predictive ability of the method proposed here is not quite as good, but considering its simplicity, prediction of postoperative pain from pain associated with venous cannulation and propofol infusion might easily be introduced into clinical anesthetic practice and used together with other methods of prediction.

We are aware of some limitations of this study. The 90-min study period of postoperative observation does not reflect pain experienced later during postsurgical recovery. However, as most of our patients have a short median time of stay in the post-anesthesia care unit after laparoscopic cholecystectomy, our study was designed to enable consistent and reliable measurements by trained post-anesthesia nurses in all patients. One could also argue that assessment of pain intensity during movement or coughing might

have been a better endpoint further improving the ability of prediction³⁹.

We did not test for psychological variables in this study. Scoring systems like the State-Trait Anxiety Inventory (STAI), Amsterdam Preoperative Anxiety and Information Scale (APAIS), or Pain Catastrophizing Score (PCS) have also been reported to be able to predict postoperative pain levels^{14,40}. Possibly, psychological factors like anxiety or pain catastrophizing might contribute to higher scored levels of pain intensity on venous cannulation and propofol infusion, considering that PCS levels have been reported to correlate ($r_s = 0.197$) with pain associated with venous cannulation with a positive predictive value of 42%⁴¹.

In conclusion, we have shown, for the first time, that preoperative bedside assessments of pain intensity associated with peripheral venous cannulation and infusion of propofol can be used to predict postoperative pain and requirements for analgesia in both men and women undergoing laparoscopic cholecystectomy. Pain induced by venous cannulation and propofol infusion is easily evaluable before surgery without specific equipment or training. Cannulation-induced pain intensity above 2.0 VAS units is associated with more postoperative pain, and with earlier and more administration of opioid, and individually obtained lower levels of cannulation- and propofol-induced pain intensity indicate lower risk of postoperative pain after laparoscopic cholecystectomy. Nevertheless, this method should also be evaluated in other surgical patient cohorts.

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Paper II



Prediction of Postoperative Pain From Electrical Pain Thresholds After Laparoscopic Cholecystectomy

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Objective: Early postoperative pain correlates to persisting pain, psychosocial distress, and delayed mobilization with thromboembolic and infectious complications. Electrical pain thresholds (EPT) have shown promising results in being able to predict postoperative pain, but the results are conflicting. The aim of this study was to test whether EPT levels can be used to predict the postoperative pain in patients of both sexes.

Materials and Methods: One hundred eighty patients scheduled for laparoscopic cholecystectomy were included in this prospective clinical study. Individual levels of EPT were measured before surgery, and the pain intensity was evaluated in the early postoperative period.

Results: There were significant correlations between EPT and the maximum postoperative pain intensity ($r_s = -0.21$, $P = 0.009$), time to the first rescue opioid ($r_s = 0.26$, $P = 0.006$), and the total dose of rescue opioid ($r_s = -0.22$, $P = 0.001$). The interaction test showed significant influence of the sex on the ability of EPT to predict the postoperative pain intensity. Female patients with low EPT (< 15) had a 4.5 times higher risk of postoperative pain ($P = 0.003$).

Discussion: Levels of EPT are reproducible, and the technique is well tolerated. However, it can be used to predict postoperative pain only in women. A weak correlation with the postoperative pain intensity, found here as well as previously, and the high sex dependency of the EPT levels obtained considerably limit the predictive value of this technique for routine use in perioperative clinical practice.

Key Words: electrical pain threshold, sex-dependent pain prediction, pain prediction, postoperative pain

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It is important to learn how to better identify in advance those patients who may experience more pain postoperatively. Starting early aggressive pain treatment in at-risk patients may reduce pain, whereas low-dose pathways may reduce side effects in others. More optimal postoperative pain relief may improve the surgical outcome, and reduce risks of venous thromboembolism and infection, by enabling earlier mobilization. There is research suggesting a link between

preoperative pain thresholds and postoperative pain.^{1–7} Various methods for estimating pain thresholds with different modalities of preoperative quantitative sensory testing, based on the induction of various kinds of experimental pain, for example with electricity,^{8–10} heat/cold,^{4,5,7,11,12} or pressure,⁶ have been reported to correlate with the postoperative pain sensitivity. Among devices for quantitative sensory testing, those developed to test electrical pain thresholds (EPT) are often handy, easy to use, safe, and reliable.^{9,13–15} However, results concerning their predictive ability for postoperative pain are conflicting, and EPT levels have been reported to correlate with levels of acute postoperative pain after cesarean section in women,^{9,16} but not after groin hernia repair in men.¹⁷ The simplicity of the method and promising results obtained in women^{9,16,18} encourages further evaluation, as also suggested in recent reviews.^{2,19}

The aim of this study was to test the ability of EPT levels to predict the postoperative pain intensity in adult patients of both sexes subjected to elective laparoscopic cholecystectomy. The hypothesis was that patients with lower EPT levels would experience more pain postoperatively.

MATERIALS AND METHODS

Ethics and Study Design

This prospective clinical observational study on surgical patients scheduled for laparoscopic cholecystectomy was carried out at 2 centers with identical anesthetic and analgesic protocols, at the county hospitals of Halmstad and Kungälv, on the west coast of Sweden, between May 2011 and May 2014. This study is a part of a more extensive investigation, and details on inclusion and perioperative management have been reported elsewhere.²⁰

The study was approved by the regional Ethical Review Board at Lund University (Dnr: 2010/391) (Lund, Sweden), and was designed and carried out in accordance with the Helsinki Declaration of 1975, as revised in 1983. The primary endpoint was postoperative pain during the first 1.5 hours in the postanesthesia care unit as evaluated with measures of pain intensity scored on a visual analogue scale (VAS), time to the first rescue opioid, and the total dose of rescue opioid.

Participants

The patients were informed about the study in writing a few weeks before the operation and orally upon arrival at the hospital in the morning on the day of surgery.

Inclusion criteria were age 18 to 80 years, American Society of Anaesthesiologists classification I to II, the ability to understand instructions, no contraindication to the premedication, and no recurrent preoperative pain (defined as pain occurring daily or requiring the regular use of analgesic drugs, or as any condition of chronic pain). Individual psychological factors were not recorded.

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Patients considered eligible for inclusion were consecutively informed and asked to participate. After oral and written informed consent, each patient was assigned a number of inclusion and was informed on how to use a horizontal VAS ruler²¹ for the assessment of pain intensity.

Study Procedures

The patients arrived at the hospital in the morning. Before they were given premedication, a specific device (PainMatcher; Cefar Medical AB, Lund, Sweden) was used to determine their EPT. This device induces monophasic rectangular electrical impulses with a constant current of 15 mA and frequency of 10 Hz when pressing with the thumb and the index finger. To yield step-wise increasing levels of pain intensity, the pulse duration increases gradually over time (from 4 to 396 μ s), thereby increasing the amount of energy delivered. When the patient releases the device, a microprocessor-controlled value of 1 to 99, reflecting the energy delivered, is shown and recorded.

Patients were first allowed to test the device once. During testing, they were asked to hold on, thereby letting the intensity of sensation increase until considered painful, and then let go. Results were shown (to the investigator only) on an LCD screen as transformed EPT score values (between 0 and 99) according to the actual maximum pulse duration. The test was repeated 3 times, and the mean value was used.^{13,14} The patients were blinded to the results.

All procedures of testing, with patients sitting upright in a comfortable position, were managed in a calm environment by trained, dedicated nurses. The clinical setting was a room in the ward or in the preoperative reception area.

Anesthetic Procedures

Premedication comprised oral etoricoxib, paracetamol, slow-release oxycodone, and ondansetron, with the addition of intravenous (IV) betametasolone immediately before the induction of anesthesia.

Anesthesia was induced IV with propofol and remifentanyl, and maintained with propofol and remifentanyl by target-controlled IV infusion, based on algorithms for the computerized estimation of appropriate plasma and target tissue concentrations adjusted to sex, age, and body weight (Alaris, CC Plus Syringe Pump using the Marsh model provided by CareFusion, Sollentuna, Sweden) in agreement with local clinical routines. Rocuronium was given IV to facilitate endotracheal intubation, and morphine (0.2 mg/kg to maximum 15 mg) for postoperative analgesia.

Surgical Procedures

Laparoscopic cholecystectomy was performed using one 10 mm and two 5 mm trocars, and the gallbladder was extracted through the 10 mm port site. Intra-abdominal expansion was achieved by insufflation of carbon dioxide to an intra-abdominal pressure of 10 to 12 mm Hg, which was then evacuated before the closure of the port sites with suture and metal clips. Ropivacaine 7.5 mg/mL (3 mg/kg) was administered for local anesthesia at the entrance holes and over the liver bed at the beginning of surgery.

Postoperative Procedures

A horizontal VAS ruler was used to estimate (by awake patients), to 1 decimal point (0.0 to 10.0), the postoperative pain intensity at rest from arrival in the

postanesthesia care unit, and at 10, 20, 30, 60, and 90 minutes. Testing was performed by trained intensive care nurses, nonblinded to previous results, but not involved preoperatively. In patients reporting values of pain intensity at or above 4.0 VAS units, defined IV rescue doses (2.5 mg) of morphine were given at 5-minute intervals until the level was below 4.0. Postoperative pain was evaluated by the estimation of the maximum pain intensity, the time from extubation to the first dose of opioid, and the total dose of opioid, during the initial postoperative 90-minute period.

Statistical Procedures

Originally a total of 151 study patients had been calculated to enable statistical confirmation (with 95% probability and 80% power) of a difference in the postoperative pain intensity of 2.0 VAS units or more between those reporting preoperative pain testing to be associated with pain intensity above or below the median level of pain.²⁰ We consider this estimation to apply also to the prediction of postoperative pain from EPT levels in this setting. A post hoc power analysis was performed to confirm this. The median level of postoperative pain in our study was 5.0 (interquartile range [IQR] 2.5 to 7.1) VAS units, and a difference of 2.0 VAS units was considered to be clinically relevant. A sample size of 152 was calculated to enable such a difference (or larger) to be statistically confirmed with 95% probability and 80% power.

Results are reported as median with the IQR in parentheses. Statistical tests for nonparametric data were used. Values of time to the first rescue dose of opioid were analyzed with the Kaplan-Meier log-rank test. Other continuous variables (EPT, postoperative pain intensity, opioid dose) were compared between groups with the Mann-Whitney *U* test. Categorical variables (sex, site, proportion given opioid) were compared between groups with the Pearson χ^2 test. Correlation coefficients (r_s) between variables were calculated with the Spearman ρ correlation test. The median level of EPT was chosen as a cut-off point for the diagnostic test and the ROC curve was used to investigate ideal thresholds. Sex differences were analyzed with logistic regression analysis and the test of interaction using the logistic regression model.

The SPSS statistical software package (IBM Inc., Armonk, NY), version 20.0, was used in all statistical analyses. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Demographics

In total, 180 patients were included in this study, and 152 patients (110 women) were available for analysis (Fig. 1). These patients were 49 (IQR 38 to 63) years old and weighed 78 kg (IQR, 70 to 90 kg).

EPTs and the Postoperative Pain Intensity

The median EPT level was 15 (IQR 9 to 22) score units with a range of 2 to 90. This level was chosen as a cut-off for prediction tests. A ROC curve for EPT to predict maximum postoperative VAS levels yielded AUC 0.64 (95% confidence interval, 0.55-0.74), and ideal thresholds were confirmed to be at the level of 15 (specificity 70% and sensitivity 62%). Patients estimated their maximum level of pain intensity to be 5.0 (IQR, 2.5 to 7.1) VAS units, the time to the first rescue dose of opioid was 20 minutes (IQR,

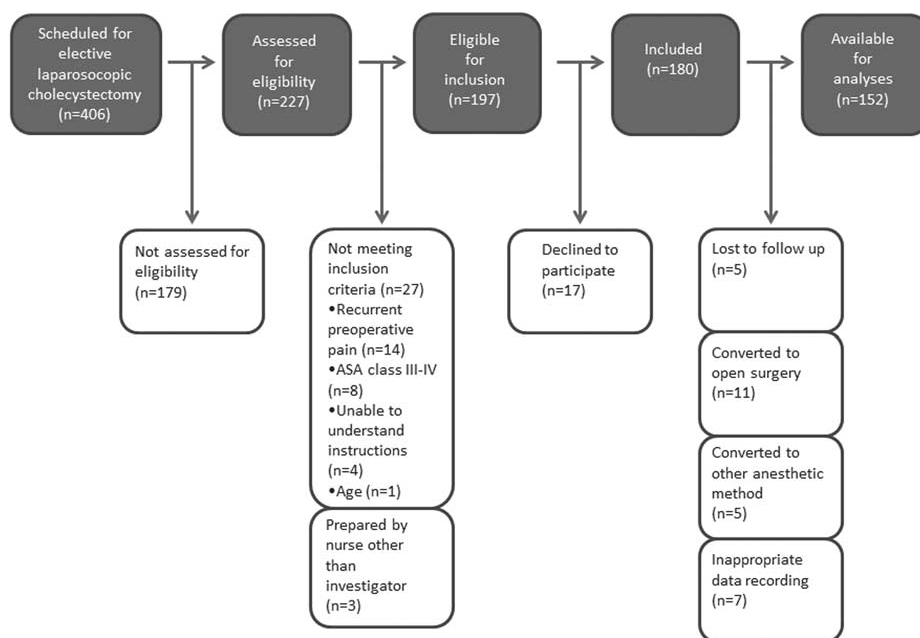


FIGURE 1. Inclusion flow chart. The reason for not being assessed for eligibility was that no investigator was on duty. ASA indicates American Society of Anaesthesiologists.

3 to 90 min), and the total dose of opioid was 2.5 mg (IQR, 0.0 to 7.5 mg), within the first 90 minutes.

There were significant correlations between EPT levels before surgery and the maximum postoperative pain intensity ($r_s = -0.21$, $P = 0.009$), time to the first dose of opioid ($r_s = 0.26$, $P = 0.001$), and the total dose of opioid ($r_s = -0.22$, $P = 0.006$) (Table 1).

In female patients, correlations between EPT levels and the maximum pain intensity ($r_s = -0.27$, $P = 0.005$), time to the first dose of opioid ($r_s = 0.30$, $P = 0.001$), and the total dose of opioid ($r_s = -0.30$, $P = 0.002$) were stronger. In male patients, there were no statistically significant correlations of EPT levels with measures of postoperative pain.

Men had higher ($P < 0.001$) EPT levels (25 [IQR 12 to 32] vs. 14 [9 to 20] score units) and were given opioids later ($P = 0.046$) than women (after 90 min [5 to 90 min] vs. 16 min [1 to 90 min]), but there were no sex differences in

the maximum pain intensity level or the total dose of opioid postoperatively (Table 2). Five patients (3 women, 2 men) estimated their postoperative pain intensity at some point to ≥ 4.0 VAS units, but were not given opioid, which explains the median pain score of 4.2 in men despite a median opioid dose of 0 mg (mean 3.8 mg).

Interaction testing showed significant ($P = 0.019$) influence of the sex on the ability of the EPT to predict postoperative pain. Therefore, multivariate regression analyses were performed separately in female and male patients. Female patients with EPT levels below the median value (15 score units) had a 4.5 times higher risk ($P = 0.003$) of postoperative pain after adjustment for age. No such association was found in men. Age seems to influence the postoperative pain more in women than in men, but does not influence the predictability of EPT levels (Tables 3 and 4).

DISCUSSION

As hypothesized, this study showed fairly good correlations between EPT levels and measures of postoperative pain in patients subjected to laparoscopic cholecystectomy. EPTs are easy to determine, and moderate correlations with all measures of postoperative pain (pain intensity, time to first opioid dose, and total dose of opioid) were obtained. However, a post hoc analysis indicated the predictability of EPT levels in women only.

Available data on the predictability of postoperative pain from EPT levels are conflicting. Our results are in

TABLE 1. Correlations Between Electrical Pain Thresholds and Measures of Postoperative Pain

	r_s	P	r^2 (%)
Postoperative maximum pain intensity	-0.21	< 0.01	4.4
Total dose of rescue opioid	-0.22	< 0.01	4.8
Time to first rescue opioid	0.26	< 0.005	6.8

r_s indicates correlation coefficient according to Spearman ρ ; r^2 , coefficient of determination in percent.

TABLE 2. Electrical Pain Thresholds and Measures of Postoperative Pain in Female and Male Patients

	Female Patients (n = 110)	Male Patients (n = 42)	P
Electrical pain threshold	14 (9-20)	25 (12-32)	< 0.0001
Postoperative maximum pain intensity (VAS units)	5.0 (3.0-7.5)	4.2 (2.2-6.5)	0.10
Time to first rescue opioid (min)	16 (1-90)	90 (5-90)	< 0.05
Total dose of rescue opioid (mg)	2.5 (0.0-7.5)	0.0* (0.0-7.5)	0.19

Values are reported as median (interquartile range).

*Mean value 3.8 mg. The median value of 0.0 is due to 2 men with VAS ≥ 4 not receiving rescue opioid and due to the distribution of values.

VAS indicates visual analog scale.

agreement with findings after cesarean section.^{9,16,18} Two of these studies^{16,18} report similar levels of correlation with the postoperative pain intensity as found here, whereas the third one⁹ reports higher correlation ($r_s = 0.65$, $P < 0.01$), but not with the postoperative use of opioid. In contrast, studies carried out on male patients have found no such predictive properties of individual EPT levels.^{8,10,17,22} It has therefore been suggested that EPT, for unknown reasons, can be used to predict the postoperative pain intensity only in women.²

We know from previous studies that women have lower pain threshold levels than men,²³ and female patients have been reported to have more pain after cholecystectomy.^{24,25} We have now confirmed this and also the clear bias of sex in the prediction of postoperative pain from individual EPT levels. On the basis of multiple measures of postoperative pain after another kind of surgical procedure carried out in both female and male

patients under highly standardized anesthetic and surgical conditions, we have confirmed that EPT levels are predictive of the postoperative pain in female,^{9,16,18} but not male,^{8,10,17,22} patients.

Predicting the postoperative pain intensity after laparoscopic cholecystectomy is most desirable considering its widespread use. Pain in the immediate postoperative period is common with high levels of pain intensity reported here as well as in numerous previous studies.²⁶⁻²⁸ Three of 5 patients have moderate or severe pain within the first 24 hours after surgery,²⁷ and up to 11% have been reported to develop chronic pain after cholecystectomy.²⁹ Postoperative levels of pain intensity are approximately the same today as 20 years ago³⁰ despite considerable clinical progress in anesthetic and surgical procedures. This highlights the need for individual evaluation of pain sensitivity and individually optimized pain management. One way to address this issue is to use quantitative sensory testing. Transcutaneous electrical stimulation—by bypassing a variety of skin receptors and directly affecting nociceptive afferent nerve fibres—has been proposed to be more reliable, reproducible, and less sensitive to technical bias than other experimental methods of pain induction.⁸ The hypothesis of reproducibility is supported by the similar preoperative EPT levels found by us before laparoscopic cholecystectomy and by others.^{9,10} The method is quite easy to apply and can be managed, as in this study, by nurses preparing patients for surgery.

There are some limitations to this study. The postoperative 90-minute study period allowed frequent and appropriate pain assessments by trained nurses. However, some patients might still have experienced higher levels of pain intensity beyond this period of time, as more intense postoperative pain has been reported—with considerable interindividual variation—within the first 4³⁰⁻³² to 6²⁷ hours after laparoscopic cholecystectomy.

We excluded patients with preoperative recurrent pain, as they were considered to have pain for other than surgical reasons, which would then be presumed to be a confounding factor. Abdominal pain might certainly be a reason for hospital contact and diagnosis. However, most of these patients experience acute pain during their episode of symptomatic cholelithiasis, which is the primary indication for elective cholecystectomy, but the clinical recommendation is surgery within 3 months after the first appearance of clinical signs and diagnosis. At the time of surgery, most patients have been free of pain for several weeks. As observed in our study, most of them had not had recurrent pain for weeks before the operation, and they were otherwise healthy. Nevertheless, these patients are known to have a higher risk of postoperative pain and should be managed with our best efforts.

Another limitation is the uneven sex distribution of the study patients, reflecting the fact that more women than men undergo laparoscopic cholecystectomy in Sweden as well as elsewhere.^{27,30} As sex analysis was not a primary objective of this study,²⁰ the sex distribution was unfortunately not a main concern. We do not consider any sex differences in variance to have influenced our results, considering that sex differences were compared in separate statistical analyses.

All investigators were female. Questions have been raised on whether, and to what extent, the sex of the investigator against that of the patient might influence the results obtained. A recent systematic 10-year review of original studies on sex and pain was unable to confirm

TABLE 3. Logistic Univariate and Multivariate Regression Analyses on the Prediction of the Postoperative Pain Intensity ≥ 4 VAS Units From Electrical Pain Thresholds in Female and Male Patients, Respectively, Adjusted for Age

	Univariate Analyses		Multivariate Analyses	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Female patients				
Electrical pain threshold				
< 15	5.2 (2.2-12.6)	0.000	4.5 (1.7-11.9)	0.003
≥ 15	1.0		1.0	
Age (y)				
≤ 40	1.8 (0.6-4.8)	0.027	0.9 (0.3-2.9)	0.246
41-59	5.0 (1.5-16.4)		2.4 (0.6-8.7)	
≥ 60	1.0		1.0	
Male patients				
Electrical pain threshold				
< 15	0.8 (0.2-2.9)	0.695	0.7 (0.2-2.7)	0.641
≥ 15	1.0		1.0	
Age (y)				
≤ 40	0.7 (0.2-7.4)	0.824	0.8 (0.2-3.3)	0.807
41-59	1.3 (0.2-7.4)		1.4 (0.2-9.1)	
≥ 60	1.0		1.0	

Values are reported as odds ratio (OR) with 95% confidence interval (CI) in parentheses. Reference levels have been set at 1.0.

VAS indicates visual analog scale.

TABLE 4. Demographics and Measures of Postoperative Pain

	EPT Level (Score Units)		<i>P</i>
	< 15 (n = 74)	≥ 15 (n = 78)	
Demographics			
Female sex (95% CI) (%)	84 (74-90)	62 (50-72)	0.003
Age (median [IQR]) (y)	43 (31-52)	56 (47-68)	< 0.001
Body weight (median [IQR]) (kg)	76 (66-90)	80 (73-91)	0.066
Duration of surgery (median [IQR]) (min)	108 (86-141)	98 (80-137)	0.243
Measures of postoperative pain			
Maximum pain intensity (median [IQR]) (VAS units)	6.0 (4.0-8.0)	4.0 (1.8-6.0)	< 0.001
Time to first dose of opioid (median [IQR]) (min)	10 (1-53)	90 (9-90)	< 0.001
Total dose of opioid (median [IQR]) (mg)	3.8 (2.0-7.5)	0.0 (0.0-5.0)	< 0.001
Proportion of patients not given opioid (95% CI) (%)	21 (14-32)	54 (43-64)	< 0.001

CI indicates confidence interval; EPT, electrical pain threshold; IQR, interquartile range; VAS, visual analogue scale.

associations between sex distribution among investigators, and pain sensitivity in men and women, respectively.³³ Nevertheless, some studies have reported both men and women to score higher pain threshold levels with investigators of the opposite sex.^{34,35} We can therefore not exclude the possibility that the sex difference in EPT levels found in our study was partly due to the fact that all investigators were women.

Dynamic assessments of pain intensity postoperatively might have been a better measure of outcome, as pain during mobilization or coughing has been reported to better reflect relevant pain,³⁶ but in this study, we prioritized frequent measurements of pain at rest. The dedicated reader might also notice that the power calculation was based on our belief that for a predictive test to be useful in clinical practice, it would have to reveal a difference of at least 2 VAS units in postoperative pain intensity to have enough clinical impact, particularly considering that a VAS is no ordinary continuous scale, and certainly not so at lower and higher levels of pain intensity. We have since then come to the conclusion that what is particularly interesting in the postoperative period is to predict who will need more analgesics, that is who is classified as having moderate (4 to 6 VAS units) or severe (≥ 7 VAS units) pain.

A strength of this study is its uniform perioperative design. We managed to standardize the perioperative procedures so that all study patients received the same baseline doses of analgesic and anesthetic drugs according to body weight. Furthermore, the systematic intraoperative use of short-acting IV drugs (propofol and remifentanyl) is unlikely to have influenced our measures of pain after surgery.

Although this study has confirmed a difference in the predictability of the postoperative pain intensity from individual EPT levels between female and male patients, it still provides no explanation for this difference. Accordingly, many studies, along with this one, have reported women to experience more pain than men,^{23,37,38} and various explanations have been proposed for differences in the pain response between sexes. Women have been reported to

have lower thresholds of pain induced by pressure, heat, cold, and electricity.^{23,39} Among proposed explanations are psychosocial factors including belief in the personal ability to tolerate pain (sex-role expectancy), coping style, catastrophizing or anxiety,^{34,40} and biological factors of genetic,³³ hormonal,²³ or psychological⁴¹ nature. Regarding the sex-dependent expectancy, men have been reported as being more prone to adjust pain threshold ratings to expectations.⁴² Anxiety and psychological stress have both been reported to predict postoperative pain and analgesic use.⁴³ Anxiety has also been found to reduce pain threshold levels^{44,45} as anxiety over anticipated test stimuli may induce hyperalgesia. In a study comparing sex differences in the pain response after fear and after anxiety,⁴⁵ anxiety did not seem to have a sex-dependent influence on primary pain testing, but rather on the sensitivity to repeated pain stimulation, where women reported more pain than men at the same level of stimulation.⁴⁶ Unfortunately, we did not include any psychological tests in this study. Anxiety seems to influence all modalities of quantitative sensory testing and we cannot rule out some influence of psychological factors or sex-dependent expectations on our findings, but if that would be the case, then similar sex differences would presumably influence all kinds of quantitative sensory testing. A proposed explanation for this sex difference in EPT is a higher nerve and receptor density in glabrous skin on the fingertips in women together with a smaller area of stimulation, leading to a higher influx of electrical energy.⁴⁷ This might explain the lower EPT levels in women, but not the sex difference in the ability of postoperative pain prediction.

Although EPT levels are easy to determine, reliable, reproducible, and well tolerated in our experience, they can be used to predict postoperative pain only in women, whereas there seems to be no predictive ability in men. Our finding of lower EPT levels in women than in men could be anticipated from previous research,³⁸ but to our knowledge, the ability of EPT levels to predict measures of postoperative pain in women, but not in men, has never been shown before in a clinical study involving patients of both sexes. The high sex dependency of EPT levels, and their low statistical correlation with measures of postoperative pain, seem to considerably limit their clinical use in perioperative practice.

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Paper III



Brief Research Report

Single Nucleotide Polymorphisms Associated with Pain Sensitivity After Laparoscopic Cholecystectomy

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Abstract

Objective. To systematically evaluate variations in single-nucleotide polymorphisms within 13 candidate pain genes in patients differing in phenotype characteristics based on a composite measure of pain sensitivity.

Methods. In a case-control study, 149 patients scheduled for laparoscopic cholecystectomy were individually categorized according to preoperative pain sensitivity and postoperative pain intensity. Cases (pain group) reported cannulation-induced pain intensity higher than 2.0, together with postoperative pain intensity of 7.0 or higher (visual analog scale [VAS] units), and controls (low-pain group) reported cannulation-induced pain intensity of 2.0 or lower, together with postoperative pain intensity lower than 4.0 (VAS units). Genotyping of exomes was performed in 32 case and 25 control patients

compared with respect to variations within 13 candidate pain genes.

Results. There were no statistically significant differences in single nucleotide polymorphisms (SNPs) within the candidate genes between the case and control groups, but minor allele SNPs in the ABCB1 and COMT genes were more common in patients with higher levels of pain sensitivity and intensity.

Conclusion. In this candidate gene study, based on a composite measure of pain sensitivity, no variations reached statistical significance after correction for multiple testing, most likely due to the large number of markers analyzed and few patients. Nevertheless, the results suggest a possible genetic contribution of single-nucleotide polymorphisms within the ABCB1 and COMT genes in individuals with higher levels of pain sensitivity.

Key Words. ABCB1; COMT; Pain Prediction; Postoperative Pain; SNP; Single Nucleotide Polymorphism; Pain Sensitivity; Candidate Pain Gene

Introduction

Despite advances in pain treatment, many patients still report moderate to severe pain after surgery [1]. More insistent therapeutic strategies are sought, including the ability to target patients prone to more pain. Prediction of postoperative pain is still a challenge, although many factors, like age, gender, pain before surgery, psychological aspects, quantitative sensory testing, and pain induced by venous cannulation, have all been shown to be influential [2–4]. Genetic factors have been proposed to explain interindividual differences in pain sensitivity and intensity [5]. A future goal in clinical settings would be to predict postoperative pain intensity—and risks of persistent pain—from individually determined pain sensitivity before surgery.

Replacement of a nucleotide in less than 1% of a population is called a mutation, and in more than 1% a single nucleotide polymorphism (SNP). A quiet allelic variation, that is, not altering the phenotype at all, may fall outside the coding region or be synonymous (inside the region but not influencing the amino acid sequence). In contrast, a nonsynonymous variation within the coding region may influence the amino acid sequence and affect the phenotype [6].

Numerous different genes and single nucleotide polymorphisms have been suggested to be involved in pain sensitivity [7]. However, it has been hard to replicate results obtained in gene association studies [8], and to our knowledge no genetic study on candidate pain genes has been designed by defining the phenotype by both preoperative pain sensitivity and postoperative pain intensity.

We have recently reported that pain intensity induced by peripheral venous cannulation correlates with postoperative pain intensity after laparoscopic cholecystectomy [2]. Blood obtained from those patients was sent for analysis by SNP array to compare SNP variations within potential candidate pain genes between a pain-sensitive group (cases) and a low-pain group (controls).

The aim of this clinical case-control study was to identify SNPs potentially associated with sensitivity of pain in surgical patients.

Materials and Methods

Study Patients

The study was approved by the regional Human Research Ethics Review Board at Lund University Faculty of Medicine, Lund, Sweden, on September 16, 2010 (Dnr: 2010/391).

A total number of 180 study patients (18–79 years of age, adequate knowledge of Swedish language, no recurrent pain) scheduled for elective laparoscopic cholecystectomy at the county hospitals in Halmstad and Kungälv, Sweden, were consecutively included after oral and written informed consents. Out of 31 patients excluded after inclusion, five were lost to follow-up, 11 were converted to open surgery, five were converted to another kind of anesthesia, and 10 had inadequate recordings, leaving 149 study patients for data analysis. Inclusion criteria and anesthetic and surgical techniques have been reported in more detail elsewhere [2].

Anesthetic Procedures

All study patients were given oral etoricoxib 120 mg, paracetamol 1500 mg (1,000 mg if <50 kg), and slow-release oxycodone 10 mg (5 mg if >65 years), for preemptive analgesia. Intravenous (iv) ondansetron 8 mg was administered, together with betamethasone 4 mg, to prevent nausea. General anesthesia was induced and

maintained by target-controlled intravenous (iv) infusion of short-acting drugs (propofol 3–4 µg/mL and remifentanyl 3–8 ng/mL) dosed according to gender, age, and body weight. Ropivacaine 3 mg/kg was used for local infiltration anesthesia in the surgical wound, and morphine 0.2 mg/kg was administered iv 30 minutes before extubation for postoperative analgesia.

Assessments of Pain Intensity

Preoperative pain sensitivity, induced by the insertion of a Venflon (Becton Dickinson, Helsingborg, Sweden) teflon cannula with a 1.1-mm inner diameter on the back of the hand, was scored on a 10.0-cm horizontal visual analog scale (VAS) at 2.0 or fewer VAS units by 70 patients, and at more than 2.0 VAS units by 79 patients (Figure 1).

Postoperative pain intensity was scored accordingly at wake-up and at 10, 20, 30, 60, and 90 minutes in the postanesthesia care unit (PACU), and the maximum levels of pain intensity during the initial 90-minute period were used as a measure of postoperative pain. Low pain (<4.0 VAS units) was found in 53 patients, and severe pain (≥7.0 VAS units) in 70 patients (Figure 1).

Phenotype Classification

We used a composite measure of pain sensitivity by scoring preoperative pain associated with venous cannulation and postoperative pain intensity after surgery. Cases (pain group) reported cannulation-induced pain intensity of more than 2.0 VAS units and high [9] maximal postoperative pain intensity (≥7.0 VAS units). Controls (low-pain group) reported cannulation-induced pain intensity of 2.0 or fewer VAS units and low [9] maximal postoperative pain intensity (<4.0 VAS units). The cutoff of preoperative pain sensitivity was chosen as previously reported in the same patient cohort [2]—patients grading their pain associated with venous cannulation below or above 2.0 VAS units have different risks of developing postoperative pain [2].

In total, 33 and 26 patients met study criteria for the case and control groups, respectively, and peripheral whole blood samples were sent for genetic analysis. However, as two blood samples, one from each study group, were lost in this process, genetic information was obtained in 32 case patients and 25 control patients (Figure 1).

Genotype Determination

The blood samples were transferred to the Department of Clinical Pathology and Cytology, Halmstad, Sweden, by permission of the Regional Biobank Centre, Lund, Sweden, for storage at –80° C within two hours of sampling. DNA was extracted to obtain 1,000 ng per sample at a concentration of 50 ng/µL in an ABgene 96 well-plate provided by SciLife Lab, Uppsala, Sweden.

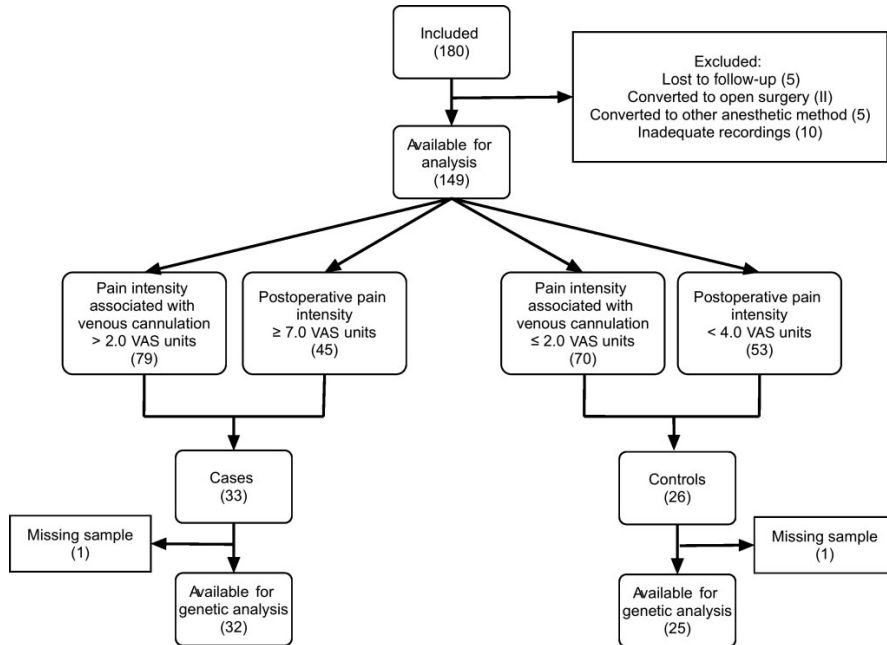


Figure 1 Inclusion flow chart. Cases (pain group), reported high cannulation-induced pain intensity (>2.0 visual analog scale [VAS] units), together with high postoperative pain intensity (≥ 7.0 VAS units). Controls (low-pain group) reported low cannulation-induced pain intensity (≤ 2.0 VAS units), together with low postoperative pain intensity (< 4.0 VAS units). Genotyping of exomes was performed in 32 case patients and 25 control patients. VAS = visual analog scale.

Sufficient concentration of DNA was verified by the Pica Green method in all samples.

Genotyping was performed according to the manufacturer's protocol using the HumanOmniExpressExome-8-v1-2-B beadchip, by the SNP and SEQ Technology Platform, Department of Medical Sciences, Molecular Medicine, Biomedical Centre, Uppsala University, Uppsala, Sweden. The total number of analyzed SNP markers was 964,193. The results were analyzed using the software GenomeStudio 2011.1 (Illumina Inc., San Diego, CA, USA) [10,11], and quality control was performed using PLINK v. 1.90b3n (<http://pngu.mgh.harvard.edu/purcell/plink/>) [12]. The assembly version Hg 19 (also known as GRCH 37) was chosen for the analysis.

In the data cleaning process, a check for gender was done as part of quality control. There were discrepancies in two samples, but both of them could be resolved by evaluation of recorded data. We checked for missing genotype rate greater than 2%, minor allele frequency of less than 1%, and sample call rate greater than 2%, and no samples were removed due to mismatch. The total genotyping rate was 0.999365. In total, 472 variants were removed due to deviation from the

Hardy-Weinberg equilibrium (threshold P values < 0.001), and 662,348 variants passed filters and quality control.

Candidate Gene Search

Our candidate genes were selected by making a search on PubMed Gene with the search terms "postoperative pain" and "homo sapiens." Thirteen genes were obtained: ABCB1, COMT, PEBP1, CYP2D6, OPRM1, CYP34A, POMC, MAOB, SCN9A, UGT2B7, SUDS3, TAOK3, and VSIG10. Functions of, and comments on, the genes are summarized in Table 1.

Statistics

Data were analyzed with PLINK v. 1.90b3n and IBM SPSS 23.0 (IBM Inc., Armonk, NY, USA) software. Associations between SNPs and the pain phenotypes were tested with linear regression analysis. To correct for multiple testing, we used the permutation option of PLINK to obtain adjusted P values (EMP2) based on 10,000 permutations for each SNP. Levels of pain intensity were analyzed with nonparametric statistics and reported as median with interquartile range (IQR). For

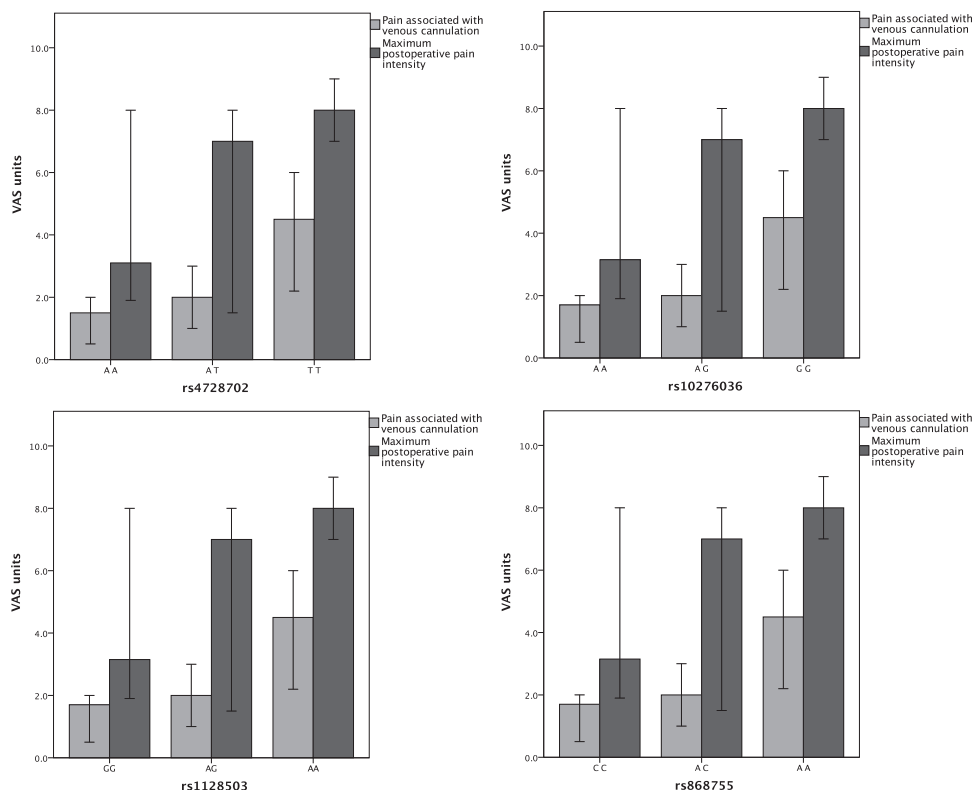


Figure 2 Distribution of pain measurements (visual analog scale units) according to single nucleotide polymorphism (SNP) genotype. The x-axis represents each SNP genotype group, with different nucleotides denoting A = adenine, C = cytosine, G = guanine, and T = thymine. The minor allele is located to the right on the x-axis. The error bars represent 95% confidence interval. VAS = visual analog scale.

proportions, 95% confidence intervals (CIs) are reported. Continuous variables like age and body weight were compared between groups with the Mann-Whitney U test, and categorical variables like gender were compared with the Pearson chi-square test. Statistical significance was set at a *P* value of less than 0.05.

Results

Patients in the case (pain) group were significantly younger ($P < 0.001$), had lower body weight ($P < 0.01$), and were more often women ($P < 0.05$) than in the control (low-pain) group. Their median pain intensity associated with venous cannulation was 3.4 VAS units compared with 1.0 VAS units in the control group, and their median maximum postoperative pain intensity was 8.0 VAS units compared with 1.5 VAS units in control patients (Table 2).

Out of 662,348 variants from the SNP array, 446 markers were identified in the 13 genes chosen as candidate genes. The 10 most significant ones are listed in Table 3. None of them reached the recommended threshold level (5×10^{-8}) for genome-wide statistical significance [13] on Bonferroni correction for multiple testing ($P = 0.00012$) or permutation testing.

As proposed by others [14], we chose a small number of SNPs with low *P* values for further visual graphical analysis (Figure 2). The minor alleles of these SNPs in the ABCB1 gene suggest a pain-sensitive phenotype both pre- and postoperatively.

Concerning a few previously well-studied SNPs believed to influence pain sensitivity [7], rs1045642 (also known as C3435T) in the ABCB1 gene has been well studied. We found no association between this SNP and pain

Table 1 Functions of 13 candidate genes assayed in blood from 57 study patients subjected to laparoscopic cholecystectomy and dichotomized with respect to individually reported high or low levels of pain intensity

Gene Gene Product	Functions	References
ABCB1 <i>P-glycoprotein</i>	Transport protein. Part of the ABC superfamily. Functional at the endothelial level in the blood-brain barrier.	[16,29–31]
COMT <i>Catechol-O-methyl transferase</i>	Enzyme. Involved in dopamine, epinephrine, and nor-epinephrine metabolism.	[15,19–26,31–34]
PEBP1 <i>Phosphatidylethanolamine-binding protein 1</i>	Monitors cell proliferation and differentiation. Involved in the production of acetylcholine transferase. Derestricted involved in the development of malignancy, diabetic nephropathy, and neurodegeneration.	[35,36]
CYP2D6 <i>Cytochrome P4502D6</i>	Involved in opioid metabolism.	[31,34,37]
OPRM1 <i>μ-opioid receptor (MOR)</i>	Opioid receptor.	[19,34,38–40]
CYP3A4 <i>Cytochrome P450 isoenzyme 3A4</i>	Involved in opioid metabolism.	[34,41]
POMC <i>Proopiomelanocortin</i>	Synthesized in various tissues. Involved in different cellular functions, i.e., pain signaling.	[42]
MAOB <i>Mono-amine oxidase B</i>	Involved in the development of allodynia in postoperative pain.	[45]
SCN9A <i>Voltage-gated sodium-channel type IV-α subunit (Nav 1.7)</i>	Sodium channel involved in pain signaling in the dorsal root ganglion and sympathetic neurons.	[43,44]
UGT2B7/ <i>Glucuronosyltransferase 2B7</i>	Involved in opioid metabolism.	[21,46]
SUDS3 <i>Suppressor of defective silencing 3</i>	Involved in early cell proliferation.	[35,47]
TAOK3 <i>Serine-threonine-protein kinase thousand and one amino acid protein 3</i>	Kinase located in the cytoplasm and cell membrane. Regulates MAPK-cascade. Could be involved in regulation of MOR signaling.	[35]
VSIG10 <i>V-set and immunoglobulin domain containing 10</i>	Viral stress inducible gene. Most intensely expressed in the spleen and placenta.	[35]

Table 2 Basic characteristics of cases and controls

Basic Characteristics	Cases (Pain)	Controls (low pain)	P
Age, median (25–75% IQR), years	41.0 (29.8–48.0)	53.0 (47.0–69.0)	0.001
Body weight, median (25–75% IQR), kg	74.0 (65.0–80.0)	84.0 (79.5–90.5)	0.004
Female gender (95% CI), %	88 (80–96)	64 (52–77)	0.036
Pain associated with venous cannulation, median (25–75% IQR), VAS units	3.4 (2.1–6.0)	1.0 (0.5–1.4)	<0.001
Maximum postoperative pain intensity, median (25–75% IQR), VAS units	8.0 (7.8–9.0)	1.5 (1.0–3.0)	<0.001
	32	25	

CI = confidence interval; IQR = interquartile range; VAS = visual analog scale.

Table 3 Result of the genome-wide array. The 10 most significant single nucleotide polymorphisms among the candidate genes with their respective gene, location, nucleotides, minor allele frequencies, and statistics

Gene	Single Nucleotide Polymorphism	Chrom	Minor/Major Allele	Minor Allele Frequency Per Phenotype		Odds Ratio	<i>P</i> -value	EMP2
				Cases (Pain)	Controls (Low pain)			
ABCB1	rs4728702	7	T/A	0.52	0.26	3.04	0.0060	0.5925
ABCB1	rs1128503	7	A/G	0.5	0.26	2.85	0.0093	0.7037
ABCB1	rs10276036	7	G/A	0.5	0.26	2.85	0.0093	0.7037
ABCB1	rs868755	7	A/C	0.5	0.26	2.85	0.0093	0.7037
ABCB1	rs11975994	7	G/A	0.5	0.26	2.85	0.0093	0.7037
ABCB1	rs1202169	7	G/A	0.5	0.26	2.85	0.0093	0.7037
ABCB1	rs1202168	7	A/G	0.5	0.26	2.85	0.0093	0.7037
ABCB1	rs1202167	7	A/G	0.5	0.26	2.85	0.0093	0.7037
COMT	rs9265	22	C/A	0.41	0.18	3.12	0.0094	0.7124
COMT	rs2518824	22	C/A	0.41	0.18	3.12	0.0094	0.7124

A = adenine; C = cytosine; Chrom = chromosome; EMP 2 = significance after permutation testing; G = guanine; T = thymine.

sensitivity (odds ratio [OR] = 0.829, $P > 0.300$). In the COMT gene, rs4680, also referred to as val158met (OR = 0.857, $P > 0.300$), and rs740603 (OR = 0.68, $P > 0.300$) did not show significant association with maximum postoperative pain intensity. Carriers of the minor allele of rs887200 reported less pain (OR = 4.98, P values = 0.028), but the results were not statistically significant when adjusted for multiple testing.

Discussion

Results obtained in this hypothesis-generating case-control study did not reach statistical significance after correction for multiple testing despite well-defined phenotypes, considerably differing in their reported levels of pain intensity. Nevertheless, specific genetic patterns brought out in these patients suggest specific SNPs to be associated with individual pain responses after surgery, in agreement with previous findings [15,16].

In this study, the most common SNPs are all located within ABCB1—an already highly presumed candidate pain gene.

Our statistically most significant SNP (significance lost after correction for multiple testing), rs4728702, has been suggested to be involved in adult antisocial behavior in a genome-wide association analysis study of a cohort with alcohol abuse [17]. Interestingly, the four most significant SNPs in our study (rs4728702, rs1128503, 10276036, rs868755) were all significantly associated with adult antisocial behavior in that study.

The ABCB1 (previously known as MDR1) gene has been implied to be involved in opioid responsiveness [7]. It encodes a drug transporter, part of the ATP

binding cassette superfamily efflux transporter, at the capillary endothelial level in the blood-brain barrier and is also involved in opioid transport. Rs1045642 (also known as C3435T) is the most studied SNP in this gene. However, we found no association between this SNP and pain sensitivity.

Catechol-O-methyl transferase (COMT) metabolizes dopamine, epinephrine, and norepinephrine to methoxytyramine, metanephrine, and normethanephrine. It influences pain sensitivity by modulating neuronal transmission [18], and polymorphisms in this gene have been reported to be associated with pain and opioid responsiveness [19–23]. Most studies have focused on a few SNPs within the gene, with various results. A frequently investigated polymorphism, rs4680 (also referred to as val158met as it codes for substitution of valine to methionine at codon 158), has been reported to be associated with pain sensitivity [24–26] and with postoperative and cancer-related requirements of opioids [20,21,27], whereas other studies of rs4680 have shown opposite results [15]. In this study, we found no association between this SNP and the pain-sensitive phenotype.

In a large study on experimental and acute postoperative pain in women undergoing surgery for breast cancer [15], 22 SNPs within the COMT gene were examined. The SNP rs2518824 was among them; however, it did not show significant results. Three SNPs within the COMT gene (rs4646312, rs2239393, rs4818) were associated with postoperative pain intensity during motion, but none of them withstood statistical correction for multiple testing, and the strongest association was between rs887200 and experimental cold pain intensity as minor allele carriers reported less pain [15]. In our

study, carriers of the minor allele of rs887200 also reported less pain, but the results were not significant when adjusted for multiple testing, and other SNPs within the COMT gene appeared to be more important. Accordingly [15], we found no association between rs740603 and maximum postoperative pain intensity, in contrast to a previous report [23].

Strengths

The phenotype was defined by composite measures including both pre-operative pain sensitivity and postoperative pain, and cases and controls well defined before high-quality genetic testing was performed by people blinded to the phenotypes. Moreover, identical management of DNA samples reduces the risk of genotyping errors and false associations [13]. This study adds new knowledge on acute postoperative pain after laparoscopic cholecystectomy to the field of genetic association studies. In the field of pain variability and genetic associations, there are studies on migraine and other chronic pain disorders and on opioid consumption [28], but there are no studies based on a composite measure of pain sensitivity.

Weaknesses

We did not use psychometric tests in this study. This would have been particularly interesting as some findings were quite similar to previous data in patients with adult antisocial behavior [17]. Moreover, we did not a priori perform a statistical power analysis for the genetic assays. Such an analysis would most likely have rendered us with a cohort size requiring a multicenter study design.

Nevertheless, the study design—based on well-defined and highly standardized clinical management of patients with respect to premedication, anesthesia, surgery, and early follow-up—might promote hypothesis-generating genetic evaluation despite low numbers of individuals, considering the opposite phenotype patterns regarding reported pain intensity. Exploratory analyses of genetic variations in small groups of patients with similar phenotype characteristics have apparent risks of error. Although the lack of significant findings challenges development of diagnostic tools for pain prediction based on this method, our findings—if reproduced in larger studies—might still be used to identify patients with higher risk of severe postoperative pain.

Conclusions

This candidate gene study in 57 surgical patients, considerably differing in phenotype characteristics with respect to reported high- or low-intensity pain, was unable to identify statistically significant differences in more than 400 SNPs in 13 candidate pain genes evaluated. Considering the large number of markers analyzed, this study is statistically underpowered, but the results also reflect the complex pathophysiology of pain. They do,

however, suggest a possible genetic contribution of single nucleotide polymorphisms within the genes ABCB1 and COMT in individuals with higher levels of pain sensitivity.

The difficulty reproducing genetic pain studies partially results from the highly complex entity of pain and possibly also from a tendency in the early days of genetic studies to almost exclusively publish positive results. To increase the chance of finding accurate connections, genome-wide association analysis can be used instead of more limited assays, especially now that they have become more readily available and less expensive. But even when comparing well-defined phenotypes, like in this study, finding a polymorphism that survives multiple statistical testing may prove to be difficult, especially considering the complex entity of pain.

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