IS CONTINUOUS PERINEURAL STUMP INFUSION OF BUPIVACAINE EFFECTIVE ON ACUTE AND CHRONIC POSTOPERATIVE STUMP PAIN? COULD IT REDUCE PHANTOM PAIN AFTER MAJOR LOWER LIMB AMPUTATION?

FINAL DEGREE PROJECT

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1. ABSTRACT

Background. Chronic pain as the result of long-term peripheral arterial disease and critical limb ischemia is difficult to manage and needs a multidisciplinary approach (pharmacological, psychological and functional) especially when the patient undergoes a lower limb amputation. Amputees show high rates of acute postoperative pain and chronic phantom limb pain in which there are few satisfactory available treatments. Nevertheless, it has been suggested for many years that continuous regional infusion of local anesthetic during immediate postoperative period is an effective and safe way of reducing these complications, morphine consumption and opioids-related adverse effects. However, data is conflicting as to its efficacy for both short-and log-term outcomes.

Objective. The purpose of this study is to investigate whether a continuous postoperative perineural infusion system with bupivacaine is effective in reduction of postoperative acute pain and morphine intake during the first 72 hours post-amputation period, and also if it has some benefice in reducing the incidence of long-term phantom limb pain and stump pain

Design. A randomized, controlled, double-blinded, multicentric clinical trial will be performed between September 2016 and May 2020 in Hospital Universitari Dr. Josep Trueta of Girona and Hospital Universitari Germans Trias I Pujol of Badalona.

Patients and methods. Patients aged up to 18 who need major lower limb amputation for irreversible critical limb ischemia as a consequence of vascular disease. Patients will be divided into two groups: one group will receive a continuous local anesthetic wound infusion treatment. The other group will receive a continuous physiologic serum infusion. All patients will be connected to intravenous morphine pump (patient controlled analgesic system) and standard treatment of postoperative pain.

Keywords. Peripheral arterial disease; Critical limb ischemia; Major lower limb amputation; bupivacaine; PCA; Post-amputation pain; Phantom limb pain.

2. ABBREVIATIONS

PAD: Peripheral Arterial Disease

ABI: Ankle-Brachial Index

CLI: Critical Limb Ischemia

MI: Myocardial infarction

DM: Diabetes Mellitus

AKA: Above Knee Amputation

BKA: Below Knee Amputation

PLP: Phantom Limb Pain

RLP: Residual Limb Pain

DRG: Dorsal Root Ganglia

SD: Standard Deviation

VAS: Visual Analogue Scale

PCA: Patient Controlled Analgesia

ISS: Inova Sedation Scale

CAT: Computerized Axial Tomography

MRI: Magnetic Resonance Imaging

TCA: Tricyclic Antidepressants

CNS: Central Nervous System

PPI: Present Pain Intensity

3. INTRODUCTION

Pain management can be complex, as there are often multiple comorbidities to be considered. In this sense, pain management for vascular surgery presents a number of challenges, including coexisting anticoagulant medication that may preclude the use of regional techniques and history of chronic pain that may determine high rates of postoperative pain and opioids consumption (1).

3.1 Definition of vascular disease and peripheral arterial disease

Vascular disease includes a wide variety of entities: Arterial [Peripheral arterial disease (PAD), renal arterial disease, and aneurysms], venous (for example varicose veins and thromboembolic disease) and lymphatic disease, Buerger´s disease, Leriche´s disease and Raynaud´s phenomenon. (1)

PAD is referred to vascular diseases which are caused by atherosclerosis of all the arteries in the body except from those which irrigate the heart and the brain. The mainly affected ones are abdominal aorta, iliac and lower extremity arteries with the consequences of occlusion and/or stenosis (2, 3). From an objective point of view, PAD is defined as an ankle brachial index (ABI) less to 0.9 (Sensitivity and specificity near 100%) **(ANNEX 1).** (4)

Other much uncommon causes of limb ischemia are Buerger´s disease (also known as thromboangiitis obliterans), vasculitis as Horton and Takayasu, and Leriche´s disease (aorto-iliac obliteration). Despite these represent less than 5% of cases of limb ischemia, the affected population tends to be younger. For example, 20-40 years old males are the most affected ones by Buerger´s disease and 35-75 years old males in Leriche´s disease.

3.2 Epidemiology of PAD

PAD is considered one of the most relevant and frequent causes of pain in lower extremity, representing also approximately 95% of the cases of limb ischemia (5). Nowadays, in the general population, PAD is presented approximately in 10-12% of the adult population (there´s no difference in prevalence between sexes). Nevertheless, in adults older than 70 years old, the prevalence increases and it is near 20%, although men and woman are still equally placed (1-2, 6). Referring to PAD incidence, the symptomatic form of the disease is related to age, increasing from 0.3%/year in 40-55 years-old patients to 1%/year in up to 75 years-old ones (3). According to Jeffrey W et al. the problem is that the symptomatic form of this disease is much less frequent than the asymptomatic one (10% of symptomatic PAD patients with the characteristic intermittent claudication versus 75% of asymptomatic patients) and consequently it is an underdiagnosed, undertreated and low understood disease by the medical community even when this kind of patient has a greater risk of death due to cardiovascular events, reason for what they must be treated (2, 6). Apart from the association to increased mortality rate because of myocardial infarction and cerebrovascular disease, another important issue involves their poor quality of life as a consequence of the disability and loss of function due to the evolution of PAD. In the old population who suffers it supposes an economic burden for themselves and also a direct economic cost for the sanitary system (3). Finally, from the point of view of the limb, it is favorable in 75% of the patients, in which claudication is stable in a 5-years perspective. Nevertheless, a group of patients (approximately 25%) will find their manifestations aggravate and 5% will require an intervention (including amputation in 2%) (4).

3.3 Risk factors of PAD

As PAD´s etiology is atherosclerotic, there is a list of risk factors that will bring on a chronic condition:

Age. Atherosclerosis is more likely to appear in people older than 40 years old and in the case of PAD, its maximal prevalence is at the ages of 65-70. (2)

Smoking.This is the most important, independent and modifiable risk factor for the development of PAD. It is a dose-response relationship (pack-year history-PAD risk). In fact, this population has more probabilities to critical limb ischemia, amputation, failure rate in bypass and minor survival rates. (2, 7)

Diabetes Mellitus. It is considered a cardiovascular risk factor, a cause of early mortality and also increases the risk of developing both forms of PAD, symptomatic and asymptomatic. In addition, it contributes (combined with poor foot care) to lower limbs amputations. This population is younger than the non- diabetics ones, affecting approximately one every three diabetics aged more than 50. (2, 3)

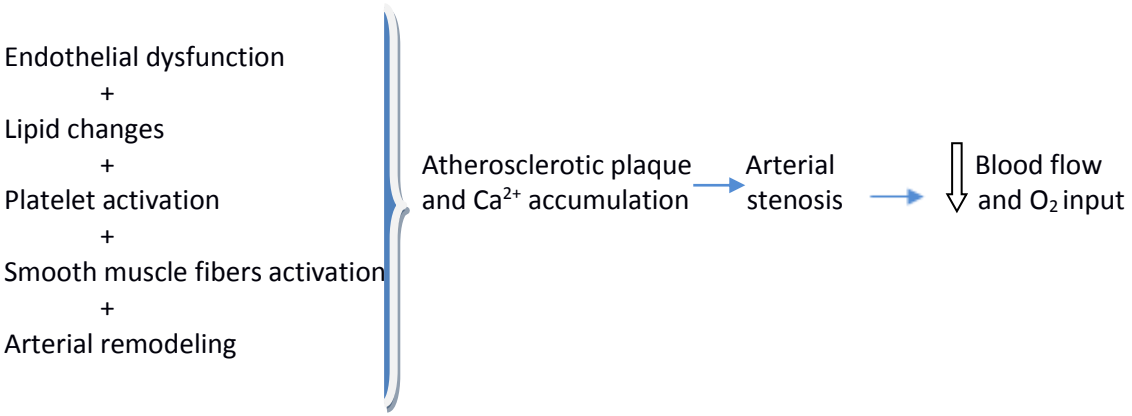
Hyperlipidemia. It is, as the previous one, a cardiovascular risk factor and it increases by 10% the possibilities of developing PAD for every 10 mg/dl rise in total cholesterol. (2)

Hypertension. It is another cardiovascular risk factor and has also a strong association with PAD. It´s also related to claudication. (2)

Others: Race, ethnicity, genetics and family history of vascular disease, chronic kidney disease, myocardial infarction, inflammation, metabolic syndrome and C-protein, β_2-microglobulin, lipoprotein, cysteine C, homocysteine levels (3, 8)

3.4 Pathophysiological mechanism of PAD

PAD consists on a degenerative process from the arterial wall in which an atherosclerotic plaque is formed: (9)



Important and independent etiologic factors of atherosclerosis are hyperlipidemia, DM, hypertension and infectious/toxic agents as tobacco (3).

Lipid accumulation differentiates atheroma from other arterial diseases, so it is considered a key element in the process. As a consequence of decrease in oxygen input, there is a decline in the arteries blood flow, reason for what muscles located distal to the occlusion cannnot get enough nutrients to their functioning (Firstly, in exercise situation, like walking, and eventually, in basal or rest situation) (4).

This kind of stenosis is mainly located in bifurcations of arteries because it is the place where there are more turbulences, shearing forces and inner coat lesion. Finally, the arteries most commonly affected are: superficial femoral and popliteal (80% of cases), iliac and abdominal aorta, and others like anterior and posterior tibial arteries (9).

3.5 Clinical presentation of PAD

Two clinical classifications have been described for PAD.

Table 1: Classification of peripheral arterial disease: Fontaine´s stages and Rutherford´s categories.

Fontaine		Rutherford		
Stage	Clinical	Grade	Category	Clinical
I	Asymptomatic	0	0	Asymptomatic
IIa	Mild claudication	I	1	Mild claudication
IIb	Moderate-severe claudication	I	2	Moderate claudication
		I	3	Severe claudication
III	Ischemic rest pain	II	4	Ischemic rest pain
IV	Ulceration or gangrene	III	5	Minor tissue loss
		IV	6	Ulceration or gangrene

TransAtlantic Inter-Society Consensus (TASC) Working Group, Management of peripheral arterial disease (PAD). J Vasc Surg. Copyright 2000.

The most common presentation symptom of PAD is pain in lower extremities. (3) The stages are usually sequential, starting with the asymptomatic form of the disease (in which physical examination and hemodynamic tests would be positive for PAD). Next stage is intermittent claudication in which patients describe muscle fatigue, pain and spasms that appear with a reproducible degrade of exercise (commonly after the same walking distance), forcing them to stop, finding relief after 15-20 minutes because of the acid lactic arrival to muscles. In this phase, pain still decreases with rest. Symptoms are distal to the occlusion: if the stenosis is located in aortoiliac arteries, the pain will appear in buttocks, hips, and tights but in case infrainguinal arteries are the occluded ones, pain will appear in calves. Finally, the critical limb ischemia appears in 1-2% of patients initially or it may be preceded by intermittent claudication. It is characterized by high intensity chronic ischemic pain that appears at night (waking up the patient), distally in toes and foot. This kind of pain is worst with cold and elevation of the limb and, on the contrary it improves with hanging down (standing, sitting on a chair, hanging the leg on a side of the bed). The extremity experiences coldness, paresthesia, hair and nails loss, and stiffness of foot and ankle joints. Furthermore, ischemic ulcerations and

gangrene may appear and because of the reduced blood flow, it is difficult their healing. In these patients, limb prognosis is particularly poor, needing revascularization and even amputations. This kind of patients (and those who present lack of cicatrizing wounds, dry gangrene or necrotizing infection) has a more important risk of limb loss without surgery, reason for what they are offered image techniques and revascularization. Finally, in case of tissue loss with infection and sepsis with hemodynamic instability it is a primary objective to discuss the possibility of surgical debridement and amputation (2–4, 9).

3.6 Treatment of PAD

Three main objectives have been established in PAD treatment: controlling cardiovascular risk factors (smoking, DM, hypercholesterolemia, hypertension…), pain management and improving symptoms and finally stopping the natural history of the disease in order to maintain the limb´s viability (4). In the first place, referring to cardiovascular morbidity and mortality, it is important for these patients to stop smoking because this action has shown decreased rates of MI, stroke, PAD progression (particularly, critical limb ischemia and amputations)(10), diet and lipid-lowering therapy which goal is LDL-C level below 70 mg/dl with statins (this is a goal for very high-risk patients and all patients with PAD are included in this group), antithrombotic therapy with aspirin (the alternative to this drug are thienopyridines such as ticlopine and clopidogrel but there is no benefit of combination therapy)(11), optimal diabetes control with HbA1c which has demonstrated a decrease in microvascular damage as neuropathy, and blood pressure management with antihypertensive therapy, mainly ACE and β-blockers in order to achieve a goal of less than 140/90 mmHg (130/80 mmHg in diabetic or chronic renal patients) (2).

Secondly, as medical treatment of claudication stands out exercise therapy (it also improves quality of life) at least 3 times per week during 30-45 minutes for a minimum of 12 weeks. It

has demonstrated more effectiveness than any of the pharmacologic treatments. Two drugs are used, cilostazol and pentoxifylline. The first one is a phosphodiesterase type 3 inhibitor which has vasodilatory properties and antiplatelet action, improving 40-60% walking distance and the second one improves red blood cell and leukocyte flexibility, increasing tissue oxygenation(2,4,9).

Revascularization is the proceeding used when there is a failure in the combination of exercise-pharmacologic treatment (after a 4-6 months trial), high limitation of the patient´s lifestyle because of the claudication, ischemic rest pain, ischemic ulcers and gangrene. This kind of procedures is based in the anatomical information provided by CTA, MRI or catheter-based angiography. There are two types of revascularization: endoscopic (angioplasty and angioplasty plus stent) and surgical (atherectomy and bypass) (2, 4).

Finally, limb amputation is performed for PAD when revascularization procedures are unsuccessful or deemed inappropriate (1). In order to avoid the maximum amputations as possible, it is essential to insist on the daily feet inspection and hygiene by the patient, mainly in diabetics one, because, as a consequence of peripheral neuropathy, they may experience a decrease of distal sensations.

3.7 Critical limb ischemia (CLI)

CLI usually occurs when two levels of the distal arterial tree are compromised and, as a consequence, the blood flow decreases so significantly that impairs the origin of collateral vessels, leading to reduction in systolic blood pressures in peripheral territories. Furthermore, there is disequilibrium between arterioles and venules pressures (the first ones are lower while the second ones are raised- explained by the inactivy of these patients and the stasis-) decreasing the perfusion without arriving to basal needs and as a result, a perpetuating death of distal tissues. The ischemic process may be also the cause of neuropathy, because once

gangrene is present, it damages sensory nerve endings preventing the patient from suffering the typical ulceration pain, and, as a consequence tissue destruction remains undetected. There are several factors contributing to this situation (*Table 2*). The only solution if this process is not controlled in previous stages is lower limb amputation (12). According to the European Consensus, CLI is defined as patients in stages IIIb and IV (end stage of PAD), which represent a 1-3% of all patients who suffer symptomatic chronic ischemia and it is associated with an impairment quality of life and increased morbi-mortality. These situations will be defined as (13,14):

17. Ischemic rest pain during more than 2 weeks + ankle systolic pressure < 50 mmHg or toe systolic blood pressure < 30 mmHg.
18. Ulceration or gangrene both in the feet or digital + ankle systolic pressure < 50 mmHg or toe systolic blood pressure <30 mmHg.

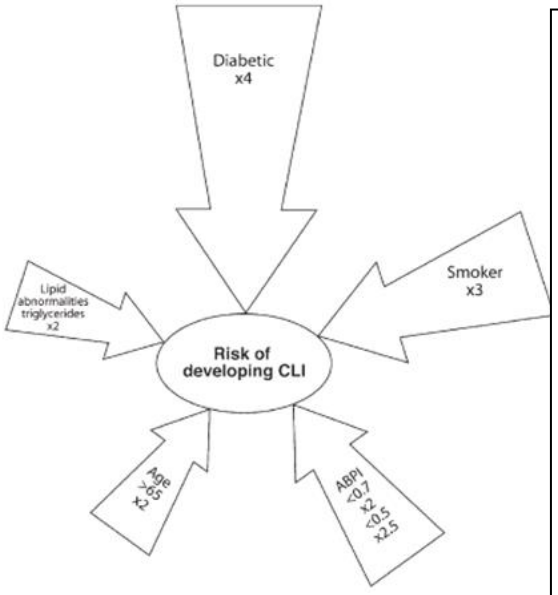
Table 2: Factors that increase risk of limb loss in patients with critical limb ischemia

Factors that reduce blood flow to the microvascular bed:
Diabetes
Severe renal failure
Severely decreased cardiac output (severe heart failure or shock)
Vasospastic diseases or concomitant conditions (e.g., Raynaud's phenomenon, prolonged cold exposure)
Smoking and tobacco use
Factors that increase demand for blood flow to the microvascular bed:
Infection (e.g., cellulitis, osteomyelitis)
Skin breakdown or traumatic injury

ACC/AHA 2005 Practice Guidelines for the Management of Patients With Peripheral Arterial Disease (Lower Extremity,Renal, Mesenteric, and Abdominal Aortic). 2006

While the ABI for PAD is <0.9, the indicative one for CLI is <0.4 (9, 14). The ischemic rest pain these patients suffer is described as "insupportable" and traduces that perfusion is not enough to maintain the viability of distal tissues. (15)

Referring to gangrene and ulcerations, the first ones commonly appear in toes and they are likely to get infected as a consequence of the decreased healing rates because of the maintained low blood flow, especially in diabetic patients, who suffer from peripheral neuropathy and the second ones are less frequent than venous ulcerations. They commonly appear in lower limbs anterior-extern zone, have a circular contour, with well delimited borders and are characterized for their deepness and pain (acute and unbearable) which may also get infected for the same reasons as toes gangrene (15). This condition is the result of ischemic alterations in lower limbs when PAD evolves and, according to S.A. Jensen et al. it is considered a serious condition if not treated. (16)



As in PAD in general occurs, prevalence of CLI is increased with age in both, men and women and it is also higher in smokers (former or current) than non-smokers. Others factors related are BMI higher than 26.3 kg/m², DM, cardiovascular events and serum triglyceride levels (*Figure* 1) (12).

Figure 1. Approximate magnitude of the effect of risk factors on the development of CLI in patients with PAD. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). 2007

The key feature in this stage of the disease is management of chronic ischemic pain, which is essential for improving quality of life and patients´ function. Nevertheless, in the natural history of the disease is shown that over 25% of CLI patients will require an amputation within the first year after its diagnosis (even when revascularization techniques, which are considered

the cornerstone of therapy to prevent limb amputation, have improved the limb prognostic) **(ANNEXES 2, 3).** (2, 16)

3.8 Lower extremity amputation

According to E. Kolossváry et al. major lower limb amputation (above the ankle) is one of the most devastating consequences of PAD (17). Patients experience phantom limb phenomena which may lead to prosthetic and ambulation difficulties, comorbidities as infections of the stump, the implicit morbidity because of the advanced aged of patients, and psycho-social problems. In addition, it constitutes a complex impact on sanitary system and health expenditure (18). As Lopez-de-Andrés et al. mentioned in their study, the mean age of patients who undergo a major lower limb amputation is 72±14 years (means ± SD) (19). For all these reasons, this kind of surgery should be limited. Its indications are: rest pain that cannot be controlled pharmacologically, infection that threatens patients´ life and extensive area of necrosis invading the foot (20). Amputations have also some objectives, which are the primary healing of the remaining stump, recovery, if possible, ambulation, and finally keeping the major extremity length as possible. (15)

Primary amputation is referred to a lower limb amputation without a previous attempt of revascularization. It is reserved for few cases because nowadays, the choice treatment for patients with severe occlusive disease is revascularization. Non-ambulatory elderly patients are particularly challenging because it´s common for them to present flexion contractures which make difficult the recovery after revascularization procedures, so primary amputation is considered an option. (20)

Secondary amputation has two main indications: the first one (60%) is unreconstructable vascular alterations and the second one is persistent infection despite of vascular reconstruction. (20)

The most performed major lower extremity amputations are below-knee and above-knee **(ANNEX 4)** and the choice is based on the level of tissue ischemia and arterial occlusion (15, 20):

19. **Below-knee amputation (BKA).** This level is preferred because of the more adequate suitability of prosthesis. In this kind of amputation, the one that preserve the knee joint, only an increase of 10% of energy is needed to ambulate with the prosthesis. Nevertheless, the healing of this kind of amputations is not really good and almost a third of the patients need a debridement, secondary close attempt or even an AKA **(ANNEX 5).** Perioperative mortality rate of BKA is 5-10%. (15,20)
20. **Above-knee amputation (AKA).** It is indicated in advanced vascular disease when there is occlusion of aortoiliac or femoropopliteal territories. The amputation is performed between the mid and inferior third of tight and the mortality is higher (15-20%) respect to BKA. Another important problem of this amputation the energy needed for ambulation, which is notably higher (65%) than in BKA and the vast majority of these patients cannot confront this effort because of their comorbidities, as advanced age, severe coronary disease, right cardiac insufficiency, chronic obstructive pulmonary disease… (15, 20).

3.9 Stump pain and phantom limb

Amputated patients suffer a lot as a result of the experienced sensations, painful or not, after their limb loss. Although in the immediate postoperative period it is really challenging to establish the difference between residual limb pain, phantom limb sensations and phantom limb pain, this contrast results more evident over time:

- **Stump pain or residual limb pain (RLP)** refers to pain located in the residual portion of the limb. It is more common in the immediate post-amputation period. Nevertheless, in approximately 5-10% of post amputated patients it persists and become even worst with time. Stump pain and phantom pain seem to be related because 50-88% patients suffer from both. In this kind of patients, physical examination of the stump commonly presents bone spurs, neuromas, infection, failure of flap closure, vascular insufficiency, adherent and wrinkled scars, so the cause of this pain is not only neuropathic but also somatic. Stump pain increases the difficulty of treatment, placing a prosthesis and rehabilitation. (21–23)
- **Phantom limb sensations**. They are experienced by most patients after surgery but not considered exactly as a problem. It consists on vivid but non-painful sensations as kinetic, kinesthetic, exteroceptive, and even feelings of movement and posture. These sensations diminish with time and they´re also related to stump pain and phantom limb pain. (21–24)
- **Phantom limb pain (PLP).** It refers to painful sensations or dysesthesia in the part of the body which has been removed (deafferentation). These sensations are described by patients as stubbing, cramping, shooting, pinching, burning and piercing, characteristics of neuropathic pain. They are commonly located in distal parts of the missing limb, where there is more innervation density and cortical representation, as toes or fingers (25, 26). The trend of this kind of pain is to be intermittent, presented in form of attacks which duration varies from a seconds to minutes and even hours.(27)

Referring to pathophysiology of PLP, it belongs to a group of neuropathic pain syndromes and both, peripheral and central mechanisms are involved (*Figure 2*, **ANNEX 6 and 7**).

The first events are the peripheral ones: after a traumatic tissue aggression, for example the nerve transection during amputation surgery, there will be a process of regenerative sprouting of the injured axons and a neuroma will be developed in the residual limb. The enlarged and disorganized endings of C-fibers ("P substance" as main neurotransmitter) and demyelinated A-fibers (Glutamate as main neurotransmitter) both in charge of transmitting mechanic, thermic and chemical stimuli to spinal cord, will show an increase of spontaneous activity. Molecular changes involve upregulation and appearance of sodium channels, decrease of potassium channel expression and the enhancement of channels sensitive to cytokines, amines which magnify nociceptive processing. Ectopic discharges from dorsal root ganglion (DRG) can help to depolarization and activation of neighboring neurons amplifying in this way the ectopic input. As a consequence, there will be an increase of sensitivity regarding neuromas and ganglia to sympathetic neurotransmitters such as epinephrine in combination to stress, temperature, and oxygenation level playing a role in exacerbation of phantom limb pain. (21, 27-28)

Focusing on central changes, there will be in both, spinal cord and supraespinal level, ending in central sensitization. In the first one, there will be long-term adaptations as a result of the sprouting nerve fibers, neuroma and ectopic discharges to projecting neurons in the posterior horn. Alterations as decrease of inhibitory GABAergic system, metabolic activity, down-regulation of opioid receptors (this one mediated by up-regulation of cholecystokinin, an endogenous inhibitor of opioid receptor) and increase of C and Aδ fibers producing structural changes in Lamina II and other areas where the synapsis occurs. In addition, receptors as Aβ which usually transmit other kind of stimuli and normally terminate in deeper laminae (III and IV) may sprout to laminae II leading to pain after innocuous stimuli. This is the mechanism known as allodynia. Brainstem, thalamus and cortex are included in supraespinal changes of phantom limb pain. (21, 27-28)

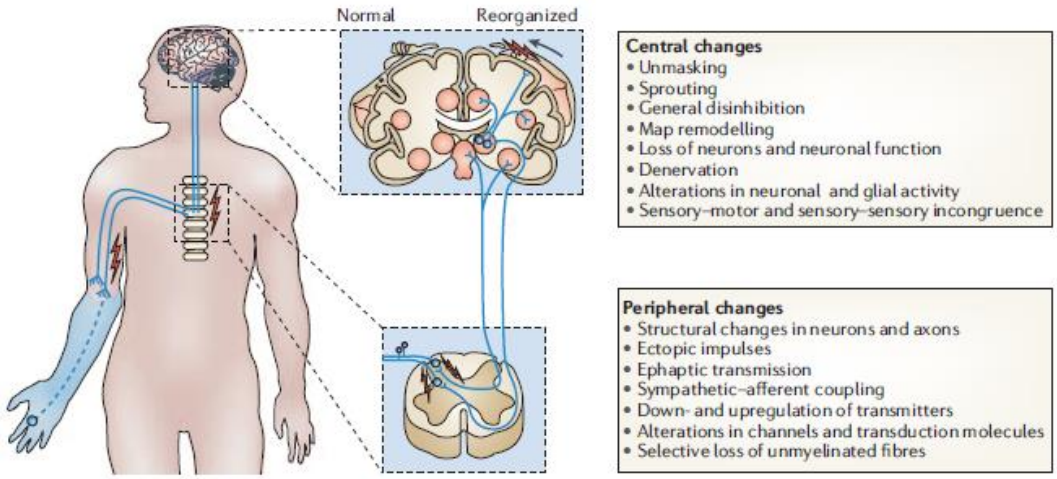


Figure 2. A schematic diagram of the areas involved in the generation of PLP and the main peripheral and central mechanisms. Phantom limb pain: a case of maladaptive CNS plasticity?. HertaFlor et Al. 2006.

The most important aspect about the "phantom pain spectrum" is its high incidence, appearing in up to 60-80% of amputated patients (21, 23-24, 27-28) and the impairments in their daily routines it causes: leading to psychological problems as depression, anxiety, and difficulties to functional recovery due to interferences with prosthesis use and rehabilitation. The onset of these sensations tends to be early, even in the first days after the surgical intervention a high number of amputees refer the feeling that the missing limb is still present or maybe specific sensations as kinesthetic ones. It progressively decreases with time, solving commonly after two or three years post amputation. Another problematic aspect about phantom pain is the inefficacy of the treatments available. Fewer than 10% of patients indicate permanent pain reliefand not all patients receive treatment (27). Therapies for these disorders should be established soon (including the perioperative management) after the onset of pain or sensations in order to prevent the structural and functional changes in both, peripheral and central structures in an attempt to avoid the chronicity these alterations suppose. Despite of all this, the treatment is difficult and it needs a multimodal approach.

3.10 Perioperative pain management for amputations: Preventing and treating acute postoperative pain, PLP and RLP

3.10 a) Preventive strategies should be based on two main aspects:

Surgical technique. It is essential a clear and proximal nerve transection. Patients could suffer an increased risk of sensitized neuroma in the distal stump, impairing the use of prosthesis if the nerve is not retracted into muscle tissue. (29) **(ANNEX 8)**

Preventive analgesia. It includes multimodal (involving medical and rehabilitative services) pre- and postoperative analgesic therapies. This approach decreases acute pain (in an attempt to prevent central sensitization and the consequent chronic neuropathic pain) and opioid consumption. The hypothesis for this kind of analgesia is that pre-amputation pain creates an imprint in CNS that may be responsible for persistent pain amputation. Other risk factors for acute postoperative pain are opioid consumption before amputation and psychological states as pre-operative anxiety. (21, 29-30)

Table 3: Perioperative pain management for amputations

Pharmacologic options	**Gabapentanoids**. Gabapentin: No studies have measured within the first 7 days following leg amputation with the administration of this drug (most studies are focused on its use for persistent PLP and RLP) Pregabalin. It seems to be effective in opioid-saving and reducing chronic pain, adverse effects must be controlled. **NMDA receptor antagonists**. The majority of studies are focused outside the immediate postoperative period. Results are mixed but ketamine seems to have benefits in this period of time. **Calcitonin**. It has been evaluated in immediate post-amputation period but due to non-randomized regimens, it´s not plausible to confirm its impact. **NSAIDS/Acetaminophen**. Both must be strongly considered in any multimodal regimen involving an amputation.

Interventional approaches	**Neuraxial analgesia**. Epidural analgesia including the appropriate moment for the catheter placement has been studied for the prevention of PLP and it is supported by several studies. **Perineural catheters.** Distal catheters. They are placed inside the transected nerve at the time of the amputation and infused during postoperative period with local anesthetic. Results are mixed regarding underlying disease, surgery and catheter location and there are barely studies of high quality. Proximal catheters. They are less studied than distal catheters in immediate pain after amputation. Further studies are needed for this objective.

Perioperative Pain Management Strategies for Amputation: A Topical Review. Pain Med. 2016

3.10 b) Treatment of PLP and RLP

The treatment can be classified in medical (which is considerate the most effective one, especially therapies with tricyclic antidepressants and anticonvulsants), non-medical and surgical. (21)

Medical treatment (21, 27)

- Tricyclic antidepressants (TCA). Amitriptyline and doxepin are drugs which inhibit serotonin and noradrenaline reuptake, leading to changes in CNS. They have proved to be effective in neuropathic and oncologic pain using lower doses than in depression.

- Anticonvulsants. Carbamazepine, lamotrigine and gabapentin act blocking non-specific sodium channels.

- Opioids. It has been suggested that oral morphine is related with a potential reduction in cortical reorganization, but tramadol, which is an analgesic with monoaminergic and opioid effect, seems an interesting alternative because of it is a weaker opioid with less tolerance and dependence problems in long-term treatments.

- Lidocaine (endovenous posology). It is a local anesthetic. It causes a reduction of nerves´ membrane permeability to sodium, and as a result of this action, there is a reversible blocking of nerve conduction.

- NMDA receptor antagonists. Endovenous ketamine´s effect is based on the decrease of the excitability mediated by glutamate (which acts in NMDA receptors) and neurokinins of spinal cord neurons, C and Aδ-afferents.

- Others: Benzodiazepines do not seem effective in phantom pain, so as NSAIDS and acetaminophen. The effects of drugs such as β-blockers, calcitonin, capsaicin and mexiletine are anecdotal as yet.

Interventional management (21, 27)

- Non-medical: The evidence of these treatments is limited. Some examples are echographic controlled phenol instillation into neuroma, pre-, peri-, and postoperative continuous sciatic nerve block, pulsed radiofrequency of this nerve, spinal cord and deep brain stimulation, stump injections, trigger point injections, blocks of sympathetic nervous system and mirror therapy.

- Surgical: Stump revision, neurectomy (if there is a neuroma), cordotomy, thalamotomy, sympathectomy…These treatments have been most abandoned nowadays as a result of their unfavorable results.

4. JUSTIFICATION

According to Herta Flor et al. in Western countries, the main reason for lower limb amputation is chronic vascular disease (28). The key feature of this process, since CLI stage to post-amputation period is chronic pain resulting in a huge impact on patient´s function, quality of life and sanitary system. This is explained because patients who undergo this kind of amputations are advanced aged and at an increased risk for postoperative complications (wound infection, MI, kidney failure) and others consequences as depression, poor physical recovery secondary from pain and ambulation difficulties, and finally impaired social satisfaction). Sanitary system expenditure includes social workers, occupational therapists, primary care practitioners and pain specialists who must face the variety of factors preventing immediate recovery and long-term functional rehabilitation (29). High perioperative and postoperative pain levels may be related to posterior PLP (being similar the degree and kind of pain), so it is essential a multidisciplinary approach (pharmacological, interventional and integrative) in both periods to diminish immediate inflammatory and neuropathic pain.

Focusing on post-amputation pain management, some studies have been done in order to prove that continuous regional analgesia with local anesthetics as bupivacaine, ropivacaine and lidocaine in early postoperative period (the first 3-5 days) is an effective technique for immediate pain management which could prevent from PLP, RLP and decrease immediate opioid requirements (and as a consequence reduce the adverse effects they have, as nausea, vomiting, sedation, delirium and respiratory depression, increased length stays at hospital and mortality) especially in advanced age patients, who are commonly the majority of lower limb amputees for CLI. The main limitations of these previous studies are insufficient number of patients, few double-blind studies, some of them aren´t randomized nor prospective, they considered a wide variety of etiologies for lower limb amputations (PAD, traumatic, oncologic…) and finally not all of them take into account below knee amputation and above

knee amputation. All these studies conclude that apparently continuous infusion of regional analgesia in early postoperative period seems a safe and effective technique and may contribute to prevent PLP and RLP but they all agree it is necessary a randomized double-blind trial with a higher population of patients in order to confirm this hypothesis, particularly at PAD amputations. (31–37)

5. HYPOTHESIS

Continuous regional perineural infusion of local anesthetic (Bupivacaine) in the early postoperative period (72 hours) through a catheter placed at the time of amputation in the sheath nerve in PAD patients is an effective and safe technique which decreases immediate postoperative pain, opioid consumption and incidence of long-term PLP and RLP.

6. OBJECTIVES

- To compare continuous perineural infusion of bupivacaine versus standardized pain management in early lower limb post-amputation period in order to evaluate immediate postoperative pain and opioid consumption with Visual Analogue Scale (VAS) and Patient Controlled Analgesia (PCA) rescue with morphine.
- To determine the effect of such interventions on the incidence and characteristics of phantom limb pain and stump pain 3 and 6 months after the surgery in the same patients.

7. METHODOLOGY

7.1 Study design

In order to avoid the limitations that previous similar studies already had, this will be a multicentric, randomized, double-blind clinical trial, particularly a phase III.

The study will be performed in Hospital Universitari Germans Trias I Pujol (Badalona) and Hospital Universitari Doctor Josep Trueta (Girona). This last one will be the reference centre but in order to coordinate the activities performed in both hospitals, a principal investigator (an anesthesiologist) and two co-investigators (a nurse and a pharmacologist) will be assigned in each center.

7.2 Study population

The study population will be patients up to 18 years old undergoing major lower extremity amputation (BKA or AKA) for irreversible critical limb ischemia as a consequence of vascular disease . During the surgery, both groups will receive spinal anesthesia. After signing the informed consent and join the trial, patients will be randomly assigned to 1 of 2 groups:
- Group I: Bupivacaine continuous infusion group through a catheter placed intraoperatively in the sectioned sheath nerve. These patients have a PCA recue with morphine. The duration of both interventions is 72 hours.
- **Group II**: Placebo group, which consists on normal saline solution in continuous infusion through a catheter placed intraoperatively in the sectioned sheath nerve. These patients also have a PCA rescue with morphine. The duration of both interventions is 72 hours.

7.2 a) Inclusion criteria:

- Patients with diagnosis of PAD, Leriche´s syndrome, Buerger´s disease or other vascular diseases particularly in its end stage critical limb ischemia, who need a lower limb amputation (BKA or AKA) as a consequence of incontrollable pain and necrotic ischemic areas with or without previous revascularization trials.
- Patients up to 18 years old.

7.2 b) Exclusion criteria:

- Patients with alterations in coagulation parameters (Essential to carry out spinal anesthesia).
- Patients unable to give informed consent.
- Patients with known allergies to any of the components of bupivacaine.
- Patients with dementia, aphasia, or any basal state in which are not able to inform VAS or manage PCA.
- Impossibility for the patient follow-up.
- Patients with dependence and/or tolerance problems to opioids
- Patients with kidney failure and glomerular filtration rate < 30 ml/min (in this patients, morphine can´t be correctly eliminated resulting in its accumulation).
- ASA 4

7.3 Sample size and sampling

7.3 a) Sample size

Sample sizes were calculated with GRANMO application©. In order to evaluate postoperative pain, this project takes into account 2 dependent variables. On the one hand, the consumption

of morphine through PCA technique, and on the other hand, the VAS, so we must calculate two different samples:

1. **PCA sample.** It is assumed an alpha risk of 0.05 and a beta risk of 0.20. According to previous similar studies, as Ayling et al. common standard deviation (SD) is 90.77 (31) and we establish as clinically significant a difference of 30 mg of morphine between patients with bupivacaine infusion and control group. Finally, as PAD population is constituted by elderly with comorbidities patients and we must follow-up the patients six months after the surgery, the estimated drop-out rate is 20%. We got a sample size of 360 patients.
2. **VAS sample**. It is assumed an alpha risk of 0.05 and a beta risk of 0.20. There is less data available for this variable but extracted from Elizaga et al. we utilized a common SD of 2.7 (30) in this scale and we established as clinically significant a difference of 4 points in VAS between bupivacaine and control group. Referring to drop-out rate, it was also assumed a 20% (same reasons as PCA sample). Finally we got a sample size of 16 patients.

After the result of both calculations, it was chosen the sample gotten with PCA data (360 patients), because it includes a higher number of patients and will be, as a consequence, more representative.

7.3 b) Sampling

A non-probabilistic consecutive sampling method will be used to recollect the patients for this study. Every time a patient who meets the inclusion criteria arrives to Hospital Universitari Germans Trias I Pujol or Hospital Universitari Dr. Josep Trueta, he or she will be informed by the anesthesiologist responsible in the corresponding center about the possibility to

participate. If they are interested, the anesthesiologist will hand the information sheet and the informed consent.

After the patient´s recruitment, this person will be stratified by age, amputation level and presence/absence of chronic pain history (in an attempt to control confounding factors) and after that, each stratum will be randomized into bupivacaine or placebo group.

7.4 Variables

7.4 a) Independent variable

Independent variable will be the use of bupivacaine or placebo in continuous infusion (ml/h) during 72 hours after major lower limb amputation surgery with a perineural catheter placed in the stump. We will consider this variable as a nominal qualitative one, differentiating between patients who have received bupivacaine or placebo.

7.4 b) Dependent variables

In order to measure postoperative pain, we have two dependent variables:

- Visual Analogue Scale (VAS). It is a pain scale in which patients range from 0 (no pain) to 10 (worst pain possible). This is a subjective method to evaluate pain but it´s essential in order to know how the patient perceives it. It is a discrete quantitative variable, so it will be expressed in means or medians depending whether it has a normal distribution or not.

VAS scores will be averaged at 1, 4, 8, 16, 24, 48, 72 hours. Furthermore, this scale will be also assessed in the 3 and 6 months following-up interview in order to calculate the rate and intensity of phantom limb pain (included in Short form of McGill Pain Questionnaire).

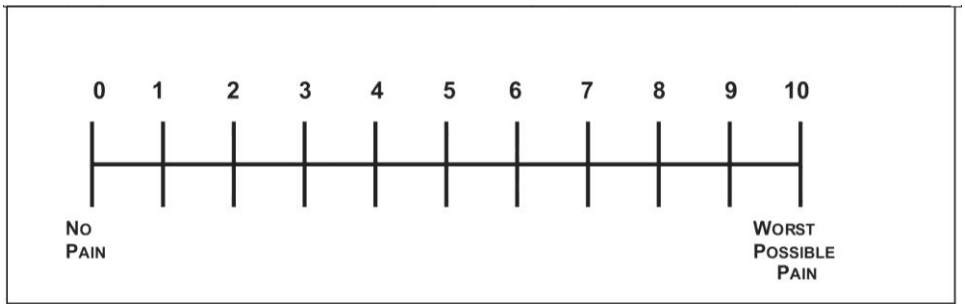


Figure 3. Visual Analogue Scale of pain.

- Postoperative opioid consumption through PCA. It is an intervention in which each patient is provided with an intravenous infusion morphine pump. This is an objective method to evaluate pain (if one patient feels more pain than another one, he will administer himself morphine more often) and to assess the morphine consumption and its adverse events during the first three postoperative days. PCA infusion (mg/h) is a continuous quantitative variable, so it will be expressed as means.

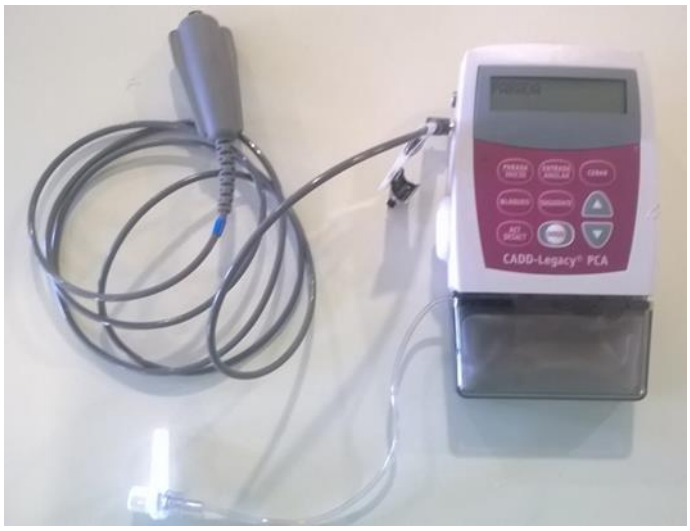


Figure 4. Patient Controlled Analgesia pump.

7.4 c) Second dependent variables

- Adverse events. Nausea, vomiting, dizziness, pruritus, delirium or difficulty voiding are adverse effects related to morphine consumption. They will be measured at 1, 4, 8, 16, 24, 48, and 72 hours. They are nominal qualitative variables expressed as yes or not.

- Sedation level. It may be a consequence of excessive consumption of morphine, so, in order to avoid this adverse event, sedation level will be monitored at regular intervals (at the same time than the rest of adverse events) during the post-amputation period. We will use Inova Sedation Scale (ISS): 1. Alert; 2. Occasionally drowsy, easy to rouse; 3. Dozing intermittently; 4. Asleep, easy to wake; 5. Difficult to wake; 6. Unresponsive. This is a discrete quantitative variable expressed as means.

- Patient´s satisfaction with analgesia. It will be recorded in the same intervals during the 72 hours postoperative period according to the following scale: 1. Very satisfactory; 2. Satisfactory; 3. Neutral; 4. Unsatisfactory; 5. Very unsatisfactory. This is a discrete quantitative variable expressed as means.

- Phantom sensations. In the 3 and 6 months phone interview, the nurse responsible (co-investigator) asked the patient to answer to a questionnaire known as: "Short-Form Mc Gill Pain Questionnaire" **(ANNEX 9).**This scale can be scored in several ways:

- "Present Pain Intensity": The adjectives are ranked according increasing intensity so each descriptor can be assigned a higher score: 0 = no pain; 1 = mild; 2 = discomforting; 3 = distressing; 4 = horrible; 5 = excruciating.

-"VAS". The scale will be integrated in the questionnaire. Patients must indicate their level of pain ranked from 0 (absence of pain) to 10 (worst pain possible).

- "Number of words chosen (NWC)": The number of words chosen by the patient.

The higher the total score on the MPQ, the more the pain experience for the patient increases. We consider this scale as a discrete quantitative variable.

- Mortality. It is a nominal qualitative variable which will be assessed by yes or not.

7.4 d) Covariates

- Age. This is one of the most influencing factors in PAD and CLI, so it is essential to stratify patients before randomization. It is a discrete quantitative variable. It will be expressed in years.

- Sex. It is a dichotomous nominal qualitative variable. It will be assessed by male or female.

- Diabetes. Diabetes is a nominal qualitative variable and it will be assessed by yes or not.

- Smoking. Smoking status will be categorized as former, current or non-smoker, so it is a nominal qualitative variable.

- Atherosclerotic disease (coronary and/or stroke). We will consider it as a dichotomous nominal qualitative variable expressed by yes or not.

-ASA scale (ANNEX 10). This is a scale used to categorize patients regarding their anesthetic risk. It is a discrete continuous variable expressed as means.

- Preoperative pain. It will be evaluated once the patient is recruited for this project. As the postoperative pain, we will measure it with VAS. It is a discrete continuous variable and it will be expressed as means.

- History of chronic pain. It is established that pre-amputation pain is related with immediate and prolonged postoperative rates of pain. Before randomization, patients will be stratified regarding history of chronic pain. It is a dichotomous nominal qualitative variable assessed by yes or not.

- Level of amputations. Our study is focused on BKA and AKA. In would be essential an equal distribution of these levels of amputations between bupivacaine and placebo group with the finality of establish if there is difference in the amount of morphine used between BKA and AKA patients, so we will stratify this variable before randomization in both groups. It is a dichotomous nominal qualitative variable.

7.5 Interventions

7.5 a) Blinding

We are performing a double-blind clinical trial, which means than neither, doctors nor patients are aware of the treatment they are giving/receiving. For achieving this, an external data manager will be hired to create the database, entrance the initial patients´ data, randomize them and register all the data collected during this study. After randomization, this person must inform the hospital´s pharmacologist in charge about the elastomeric pumps with bupivacaine or placebo that must prepare, and he/she, after setting these has to keep them into a bag with the identification number assigned to the patient (data manager and pharmacologists assigned in each hospital will be the only people who know which patient receive which technique). Vascular surgeons will be handed the pumps closed in the bag with an identification number just before the amputation and the reference anesthesiologist from each hospital, who will inform the patients about the trial, explain PCA and VAS use and recollect their data during postoperative period but they won´t know if they are in Group I or II. Finally, the nurse in charge of the 3 and 6 months phone call follow-up, won´t know either which group the patient belongs.

7.5 b) Randomization

Patients who meet the inclusion criteria will be asked to participate in this project. As the amputation in these casesis a deferrable urgency, which means it can wait one or two days, we will give the information sheet to these patients and answer to possible questions that the patient comes up with. If they are interested in participating, it´s essential to obtain their written informed consent. To avoid the selection bias, the randomization sequence will be performed by an external manager data, computer generated with SPSS after stratifying for age, history of chronic pain and level of amputation in a 1:1 ratio of bupivacaine and placebo in

order to make both groups as similar as possible and each patient will receive an identification number to guarantee protection of their personal information.

7.5 c) Intraoperative procedures

All amputations will be performed under subarachnoid anesthesia. The spinal block will be carried out with 10 to 12 mg of hyperbaric bupivacaine 0.5% at L2-3/L3-4.

All patients will be implanted a commercial catheter system for continuous wound infusion (painfusor catheter, Baxter) **(ANNEX 11, 12).** The surgeon will receive from the hospital´s pharmacy the infusion device with the drug/placebo already prepared in a closed bag without knowing if it contains saline solution or bupivacaine and will place the catheter through the skin under the fascia alongside the wound. If it is an AKA, the catheter will be located in the sciatic nerve, and if it is a BKA, the catheter will be located in the posterior tibial nerve. All internal sutures will be loose enough to allow easy withdrawal of the catheter at the end of its use. To patients in group I the infusion system was filled with 360 ml of 0.25% bupivacaine. Patients in group II the infusion was filled with 360 ml of physiological serum.The wound infusion is going to start at a rate of 5 ml/h at the end of intervention. The catheter will be routinely removed on the third postoperative day (after 72 hours). The reason for that choice is that multiple studies have shown the maximum pain is within the first 2 days after surgery.

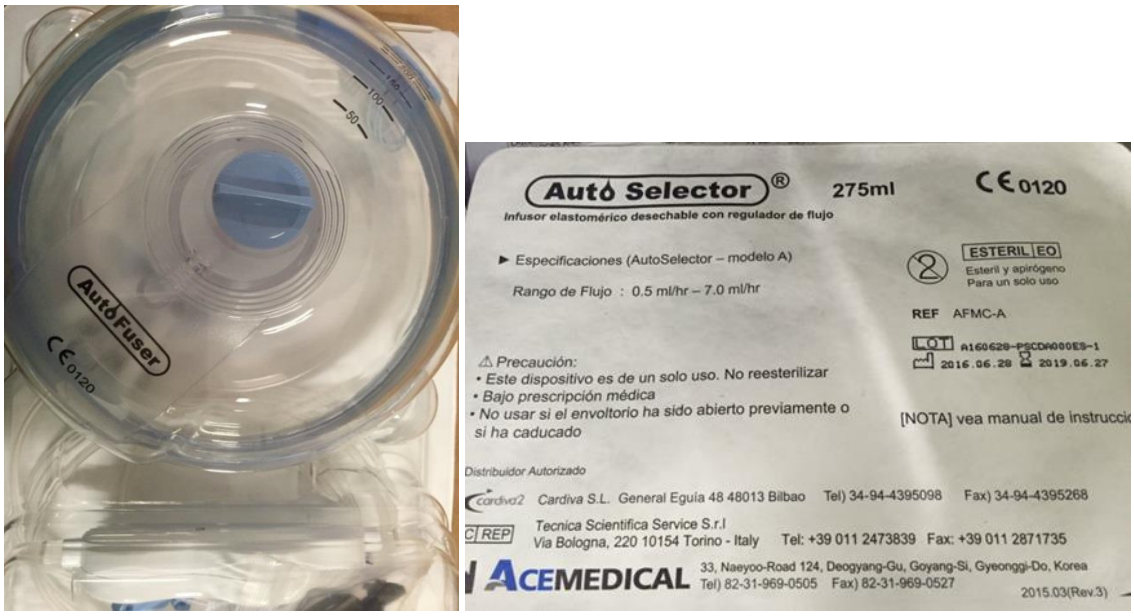


Figure 5. Elastomeric bomb for continuous infusion of local anesthetic

7.5 d) Immediate postoperative procedures

All patients will receive during post-amputation period (72 hours) paracetamol 1 gr endovenous/6 hours and metamizole 2 gr endovenous /8 hours. In addition, all patients will receive endovenous morphine via PCA. Every patient who signs the informed consent is instructed on the use of PCA. The endovenous morphine PCA pumps will be set to deliver 1-mg boluses on demand with a 5-min lockout interval and a maximum of 6 mg/h (we establish a lockout interval in order to avoid adverse events derived from an excessive morphine infusion).

Pain level (VAS), morphine cumulative doses (mg) number of times the patient press the morphine infusion bottom when the device is lockout, sedation level scale (ISS), and adverse effects will be measured at 1, 4, 8, 16, 24, 48 and 72 hours postoperatively.Stump pain scores will be evaluated at resting and coughing. Patients will be hospitalized in the reanimation plant where pain control is better established by anesthesiologist and trained nurses.

7.5 e) Clinical follow-up

After the 72 hours post-amputation period the stump catheter and PCA device will be withdrawn. Patients will continue with the analgesia that the facultative considers adequate. In order to complete the study´s objectives, we need to check the patients after 3 and 6 months. A nurse assigned in each hospital involved in the project, will phone the patients in order to administer the Short-form McGill Pain Questionnaire. If patients don't respond to the phone call, the nurse will perform a second phone call to a contact relative that previously had facilitate his/her number. If this person doesn´t answer the phone either, it necessary to check if the patient has died (especially given the advanced age and comorbidities of our population), so the nurse will address to "Índice Nacional de Defunciones" (Spain) and introduce ID, first name, last names and birth date from the patient. If the patient has died or we aren´t able of localize him/her, we will consider the fact as a drop-out.

7.6 Data collection

The decision of performing a multicentric clinical trial is based on the number of patients. If we only consider Hospital Universitari Dr. Josep Trueta, the process of data collection would be too long, so we must distribute the patients and for that reason, another hospital from Barcelona will participate.

Referring to patient information, we recorded: age, sex, level of amputation, presence/absence of previous chronic pain, patient´s comorbidities as smoking, diabetes, and atherosclerotic disease (coronary and stroke),ASA, preoperative and postoperative reported pain scores (VAS),patient´s satisfaction with analgesia, morphine cumulative dose of each patient (mg), number of times the patient press the release-morphine bottom and the device is lockout and adverse effects derived from bupivacaine and specially morphine (nausea, vomiting, pruritus, respiratory depression, delirium, sedation level), mortality and finally, data

related to phantom limb sensations and pain in the 3 and 6 months interview. A sheet for data collection will be designed by data manager.

Previously to the trial's start, all the vascular surgeons from the 2 hospitals involved will be instructed on the placement of this type of catheters to prevent the availability of the surgeon from being a bias (if only one in each department could perform the technique, the study would also increase its length). Nevertheless it is important to highlight that vascular surgeons from Hospital Universitari Dr. Josep Trueta had been previously formatted in Hospital Universitari Germans Trias I Pujol, so they share the vast majority of techniques both departments use. The anesthesiologists from both hospitals involved must also attend to the session to clarify and insist on the importance that all these patients undergo spinal block instead of general anesthesia or epidural anesthesia. After all the process of data collection, an external statistical will analyze it.

8. ADVERSE EVENTS

Presence of adverse effects during surgery (caused by spinal injection of bupivacaine) or during the postoperative period (caused by local infusion of bupivacaine or PCA endovenous morphine) will be recorded and reported by the investigators (the anesthesiologists in charge of the project in both hospitals). Our first concern must be the patient´s safety so we will act according to this, providing medical intervention. Nevertheless, the responsible investigators will remain alert to adverse events since the beginning of the trial.

8.1Bupivacaine(38)

It is really uncommon to experience allergic reactions to local anesthetics of this type. The main problem is consequence of high concentrations, up to 1´6-2 mg/L of bupivacaine in blood, especially in deteriorated patients and chronic renal or hepatic disease. Manifestations

range from mild to severe intoxication, involving mainly central nervous system (CNS) and cardiovascular system alterations.

8.2 Morphine (39)

Morphine has similar adverse effects than other opioids, but the frequency is higher, particularly in advanced age and deteriorated patients. The most frequent adverse events are gastrointestinal (nausea, vomiting) and CNS (somnolence, disorientation, euphoria, and tolerance with prolonged treatments). Others effects we must pay attention to are pruritus and respiratory depression. Anaphylactic reactions are also uncommon in this kind of drugs. In order to monitoring sedation level, we will administer Inova Sedation Scale (ISS).

9. STATISTICAL ANALYSIS

Statistical analysis will be performed with Statistical Package for the Social Science (SPSS) software for windows.

Univariate analysis

The results will be expressed in qualitative variables as means ± SD or medians depending on whether they are normally distributed or not. Referring to categorical variables, they will be expressed as percentages.

Bivariate analysis

We must compare our independent variable, considering it as a nominal qualitative one (bupivacaine or placebo continuous infusion) with each one of the dependent variables:

- In the case of PCA, it is a continuous quantitative variable. As previous studies before ours, we must convert morphine mg to mEq, getting as a consequence a normal distribution, so we will need Student´s t test.

- In the case of VAS, it is a discrete quantitative one variable, so in order to compare it with the independent one, we will need the statistic test Mann-Whitney. In order to correlate the results from VAS and PCA, we will apply Kappa Correlation Index.

Multivariate analysis

In addition, multivariate analysis will be performed with the finality of introducing possible covariates and confounding factors. The analysis will be done with logistic regression adjusted for all these variables.

In conclusion, these analyses will result in expression of Relative Risks with 95% confidence interval and it will be considered as statistically significant when p value<0.05.

10. ETHICAL ASPECTS

In order to perform this project, we need previously the evaluation and approval of the Comitè Ètic d´Investigació Clínica (CEIC) from the reference hospital where it will be executed: Hospital Universitari Dr. Josep Trueta (Girona). Furthermore, the clinical trial must be approved by AEMPS, registering in its webpage through the EudraCT application.

This study has been designed following the Ethical Principles for Medical Research Involving Human Subjects stated by the World Health Association (WMA) in the Declaration of Helsinki from 2013.

According to the principle of autonomy, an information sheet **(ANNEX 13)** will be handed to the patients who meet the inclusion criteria. This sheet will be written in a comprehensive language to make sure patients understand the project. Nevertheless, if they have any doubt, there will be phones numbers to help solving them. To include a patient in the study, it is essential to obtain their informed consent. **(ANNEX 14)**

All data collected for this clinical trial will be kept confidentially in agreement with "Ley orgánica15/1999, de 13 de Diciembre, de Protección de Datos de Carácter Personal" and with the finality of ensuring the anonymity, each patient will be assigned an identification number. The study must also respect: -

- RD 1090/2015, de 4 de diciembre, regulan los ensayos clínicos con medicamentos, los Comités de Ética de la Investigación con medicamentos y el Registro Español de Estudios Clínicos.

- RD 1/2015, 24 de Julio, ley de garantías y uso racional de los medicamentos y productos sanitarios.

- Ley 14/2007, 3 de Julio, de investigación biomédica.

We decided to incorporate a placebo group in order to confirm the efficacy of bupivacaine in local continuous infusion, because until now, it has been suggested for other studies but never confirmed. Nevertheless, none patient will be unprovided of analgesics during this postoperative period.

11. STUDY LIMITATIONS

Firstly, referring to the sample size, phase III clinical trials include up to 500 patients, so the size could be insufficient. Another inconvenient about sample size is the strong difference between calculating it according to PCA technique or VAS. Nevertheless, to overcome with the possible biases resulting of not arriving to the minimum number of patients for a phase III, these were randomized.

In order to prevent that the placement depends on the attending surgeon, all the vascular surgeons from the 2 hospitals who performed this kind of amputations will be instructed on this technique previously to the start of the trial and randomized in order to prevent inter-surgeon variations.

Another limitation is that this study will be performed in Catalonia, particularly in 2 hospitals from this Community, so it could be difficult to extrapolate final results to other regions or types of populations.

As the sampling is non-probabilistic, a selection bias could happen. This would be reflected because maybe patients who have suffered most pain show more concern about their condition and interest about this trial than patients who felt less pain (maybe because their pain threshold is higher).

Referring to the investigators availability, the most important limitation would be the anesthesiologists. We named 2 anesthesiologists, one in each center who are responsible of informing the patients, explaining the instructions of PCA and VAS, and controlling their postoperative periods but if some day they can´t attend or there are too many patients in that moment, they must get some help. If this case arrived, they as main investigators of this study must assign someone to help them punctually.

Finally, another limitation to this study is the loss of patients follow-up. In an attempt to compensate this loss, we already considered it in the sample calculation (we estimated 20% of drop-outs instead of 10% as it´s commonly consider). The main reason for this is we are dealing with an elderly and with-comorbidities population and the follow-up will last until 6 months after the surgery, so we must contemplate not-answered phone calls and deaths. This may difficult especially the long-term objective related to phantom limb.

12. FEASIBILITY

This clinical trial will be performed at 2 hospitals, 1 located in Barcelona (Hospital Universitari Germans Trias I Pujol) and one in Girona (Hospital Universitari Dr. Josep Trueta). Before the study starts, it is important to organize a meeting in the reference center in order to explain properly objectives, what we need for data collection, how to place the stump catheters, the anesthetic technique performed intraoperatively and how the trial will be developed. Despite the reference center is Trueta Hospital, the study will be carried out by staff from both of them. The necessary personnel will be:

- An external data manager (database creation, randomization, and entrance of patient data)
- 2 anesthesiologists (inform patients who meet inclusion criteria, explain PCA and VAS and control postoperative period)
- 2 nurses (follow- up phone calls)
- 2 pharmacologists (preparation of elastomeric bombs)
- An statistical (data analysis)
- Vascular surgeons from each hospital vascular department.
- Anesthesiologists from both hospitals (performing the spinal blockage in the surgery).

Data manager and statistical will be provided with informatics equipment and the possibility of working in the hospital during this period of time.

The anesthesiologist will be the person in charge of talking to the patient in the first place about the clinical trial, handing him/her the information sheep, solving their doubts (both in the moment and providing the person a contact number in order to get help when needed)

and explaining the use of PCA device and VAS. Referring to material, catheter, anesthetic drugs and normal saline solution, will be available in each hospital and prepared by nurses (for example the drug regimens after surgery) or by the pharmacologist assigned (preparations of catheters). On the contrary, we will buy the elastomeric pumps because those available in our hospital don´t have enough capacity for the 72 hours infusion.

Finally, according to estimated period of data collection, we established approximately 2.5 years after calculating number of amputations which fulfill the inclusion criteria in the hospitals participating and the 3-6 months following interval:

- Hospital Universitari Dr. Josep Trueta of Girona (Reference center): 70 amputations/years

-Hospital Universitari Germans Trias I Pujol of Badalona: 110 amputations/year

Data collection will be developed between January 1st 2017 and June 31st 2019.

13. WORK PLAN AND CHRONOGRAM

Investigators: 2 anesthesiologists (one in Hospital Universitari Germans Trias I Pujol and the other one in Hospital Universitari Dr. Josep Trueta).

Co-Investigators: 2 nurses and 2 pharmacologists.

Collaborators: Anesthesiologists and vascular surgeons from both hospitals.

Other workers: An external data manager and a statistical.

This study will be developed in a period of 45 months (approximately 3 years and 8 months) and it will be divided into 4 phases:

First phase: Preparation and coordination(4 months)

- **Protocol design**. This step will be assumed by the investigators of this project and it will take one month: reading bibliography, establishing hypothesis, objectives, defining the population and deducing how much help they will need for the interventions.

- **Initial meeting.** After this month of planning the protocol, the investigators will meet the four co-investigators assigned and also the head of section from anesthesiology and vascular surgery from both hospitals in order to explain the protocol and coordinate the centers.

- **Chronogram.** It will be designed after the agreement between the hospitals.

- **Authorizations.** Previous to start the clinical trial, we must register it in AEMPS webpage through eudraCT application in order to obtain the nº solicitude. Once this step is done, we need to get the Ethics Committee approval from the reference hospital (In this case, Hospital Universitari Dr. Josep Trueta).

- **Second meeting.** The first step will be to organize another reunion in which investigators and co-investigators have the objective to explain how to recruit the patients, what will be done exactly in the intervention and the follow-up and finally what data and how to collect them. This meeting is programmed for the external data manager, vascular surgeons and the anesthesiologists from both hospitals.

- **Database creation by the manager**

Second phase: Data collection (2.5 years)

- Recruitment of patients, intervention and follow up. This stage is linked because the sampling is consecutive non-probabilistic, so the process will be continuous: informed

consent, randomization, amputation, 72-hours data collection in postoperative period and the 3 and 6 months phone call to complete the follow-up.

- **Data collection.** During this whole process, the two main investigators (one anesthesiologist from each hospital) will be controlling the post-amputation period and collecting all the data established in the protocol. At the same time, the co-investigators nurses will also collect the results from McGill questionnaire. Every month, they will pass the resulting sheets to the data manager in order to entrance it into the database. With the same frequency, the information from the database will be reviewed to ensure it is been doing correctly.

Third phase: Statistical analyses and results interpretation (4 months)

- After the database closure by the external data manager, it will be passed to the statistical who works in the reference hospital.
- The statistical is conceded a period of time to perform the analysis.
- Finally, the statistical will sent these results to the main investigators, in order to interpret and discuss them

Fourth phase: Publishing and disseminating the results (7 months)

- Investigators will write the corresponding articles in order to show the results and publish them in anesthesiology journals.
- The second way of disseminating the results will be through the XXXVI Spanish Anesthesiology Congress (May 2020)

13.1 CHRONOGRAM

Year	2016				2017	2018	2019							2020	
Months	Sep	Oct	Nov	Dic	Jan-Dec	Jan-Dec	Jan-Jun	July	Aug	Sep	Oct	Nov	Dec	Jan-Apr	May
First phase: Protocol preparation and coordination (Inv, co-inv, data manager)															
Protocol design															
Initial meeting															
Chronogram															
Authorizations															
Second meeting															
Database creation															
Second phase: Recruitment, intervention and follow-up (Inv, co-inv, vasc surgeons, anesthesiologists, data manager)															
Patient recruitment															
Intervention															
Follow-up															
Data collection															
Third phase: Statistical analyses and discussion of the results (statistical, investigators)															
Analysis															
Discussion															
Fourth phase: Results publishing and disemination (Investigators)															
Final report															
Publishing															
Congress															

14. BUDGET

Firstly, referring to the personnel, vascular surgeons and anesthesiologists who participate in the intraoperative part of the trial already work at the hospitals involved. The two pharmacologists will take part during workable hours, so we don't need to hire anyone for this labor. Nevertheless, although the nurses involved are co-investigators, they will do extra-work in the follow-up: they must locate 360 patients 3 and 6 months after the surgery (and even patients´ relatives if the patients don't answer the phone in the first place). We estimate 100 hours between both nurses (35 in Hospital Universitari Dr. Josep Trueta and 65 in Hospital Universitari Germans Trias I Pujol of Badalona) so these nurses will receive an extra-payment. With the statistical the situation is similar, because despite he works in our reference hospital (Dr. Josep Trueta), he will probably need some extra-hours apart from his workable timetable (we consider approximately 30 extra-hours). Finally, we will hire an external data manager (this person will create the database and he will work 1 day/month during 3 hours each day in order to entrance all the data recollected during 2.5 years).

In the second place, the only material we must pay are the elastomeric bombs because we need them to have minimum 360 ml of capacity and the ones we have in both hospitals participating in our study have 275 ml maximum.

Finally, established taxes are AEMPS authorization, publishing and inscription price for Spanish Congress of Anesthesiology. Other taxes are accommodation and travels.

	Cost	Quantity	Total
Personnel			
Data manager	35 €/h	90 h	3.150 €
Statistical	30 €/h	30 h	900 €
Nurses	15 €/h	100 h	1.500 €
Material			
Elastomeric bombs	30 €	360 units	10.800 €
AEMPS authorization	1.500 €	1	1.500 €
Publication and dissemination			
Anesth. Journal	1.500 €	1	1.500 €
Anesth. Congress	500 €	2	1.000 €
Accomodation, travels	300 €	2	600 €
TOTAL			20.950 €

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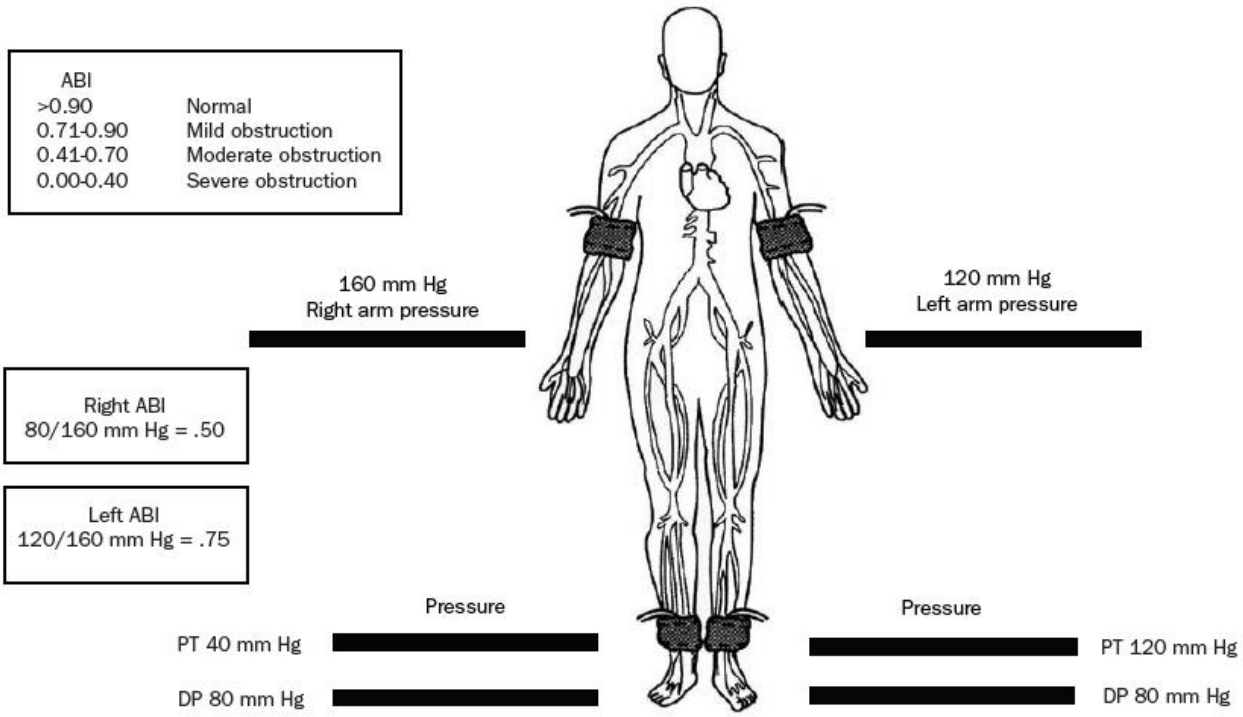
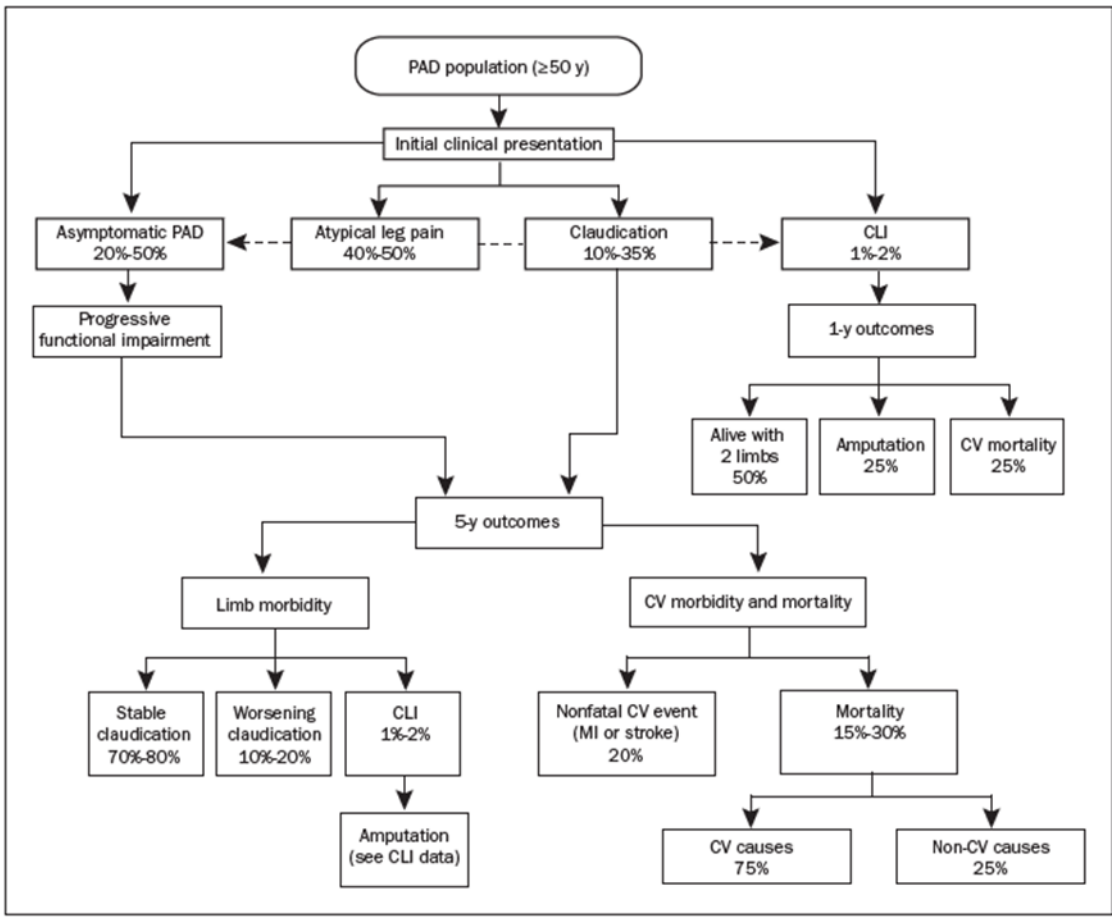
16. ANNEXES

ANNEX 1: ANKLE-BRACHIAL INDEX (2)



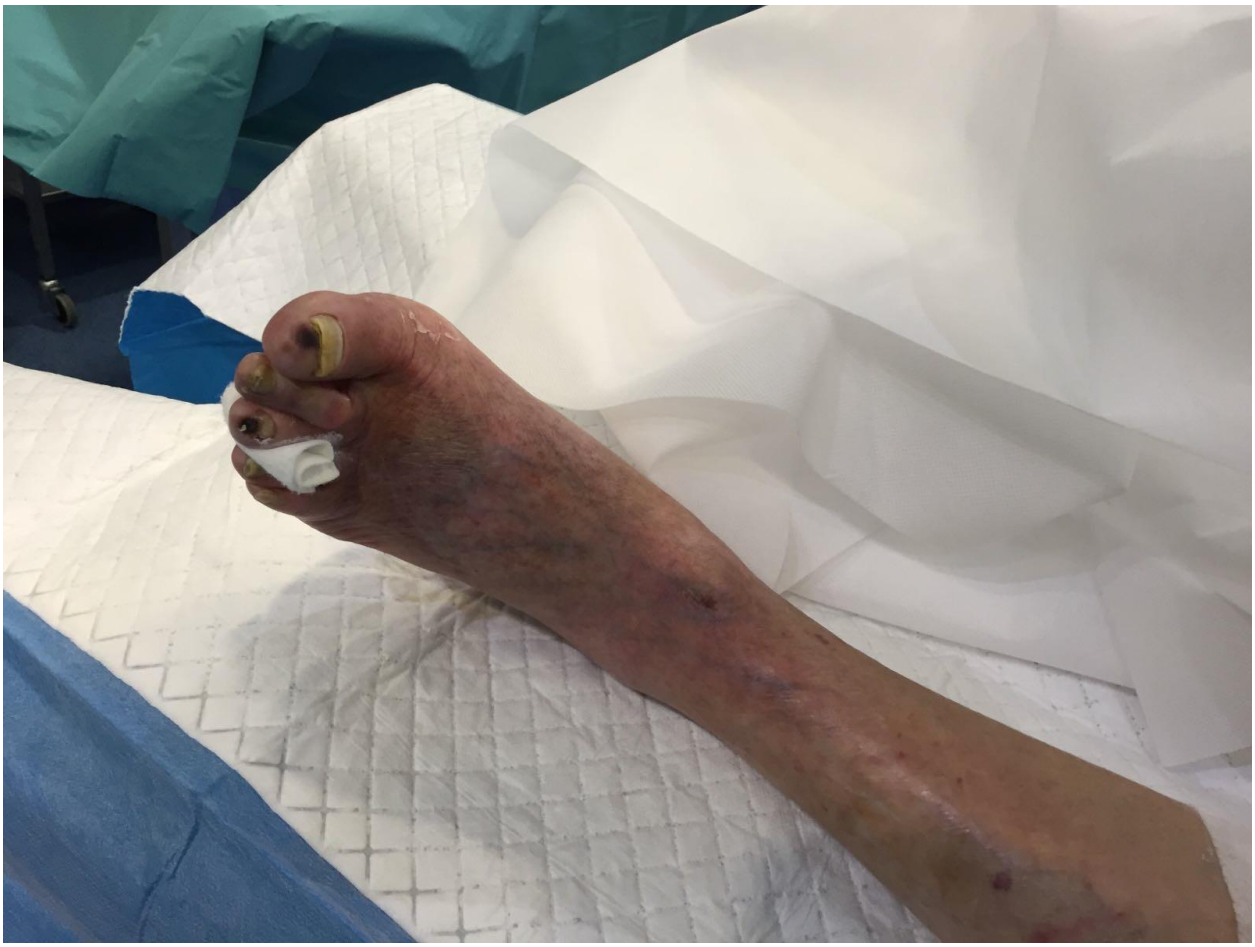
Dp= Dorsalis pedis; PT= Posterior tibial artery

ANNEX 2: NATURAL HISTORY OF PERIPHERAL ARTERIAL DISEASE (12)



CLI= Critical limb ischemia; CV= Cardiovascular; MI= Myocardial infarction

ANNEX 3: LIMB WITH IRREVERSIBLE CRITICAL ISCHEMIA BEFORE AMPUTATION



ANNEX 4: BELOW AND ABOVE-KNEE AMPUTATIONS (15)

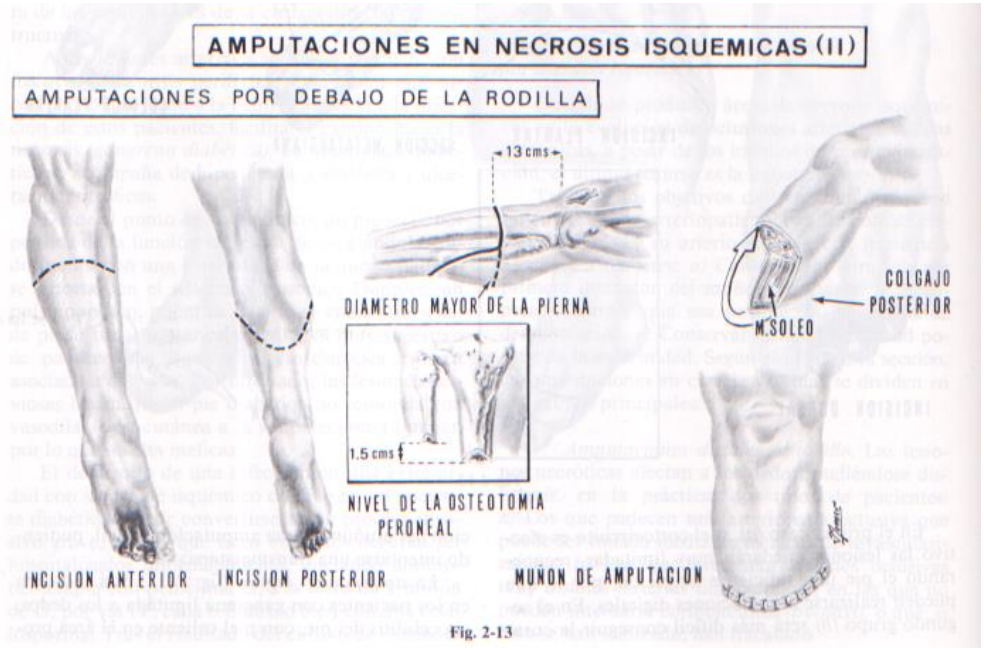


Fig. 2-13

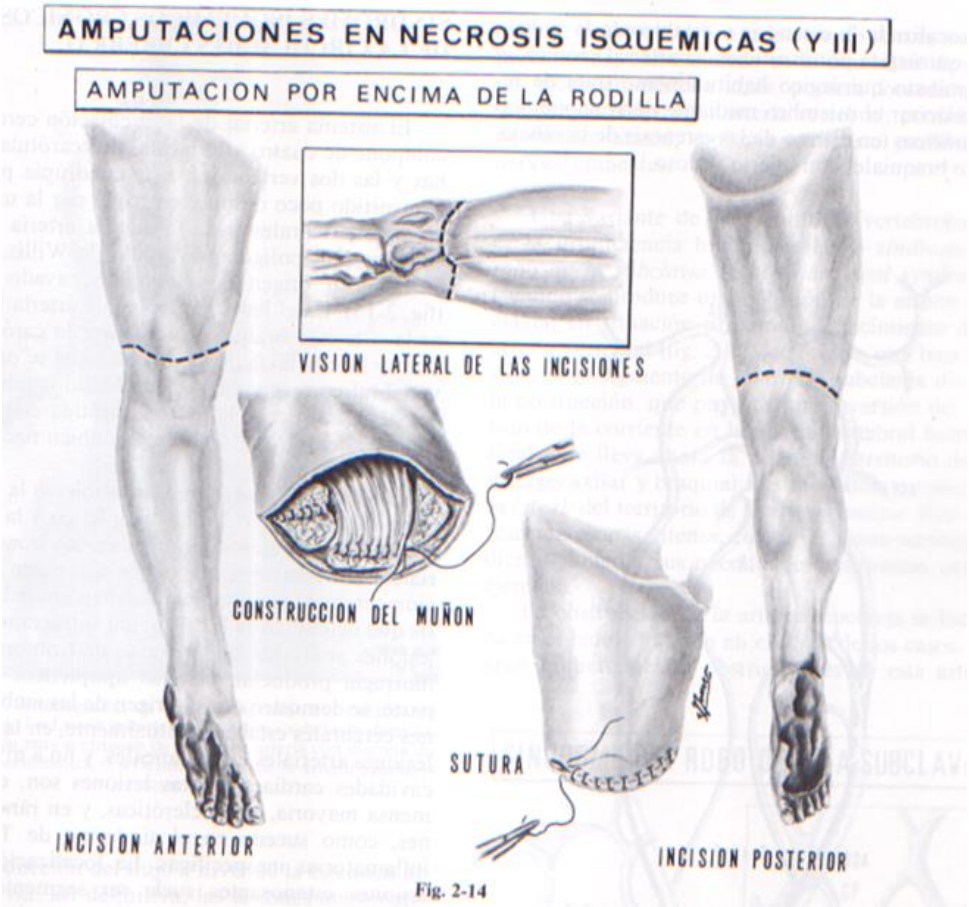


Fig. 2-14

ANNEX 5: FATE OF THE PATIENT WITH CLI AND FATE OF THE PATIENT AFTER BKA (20)

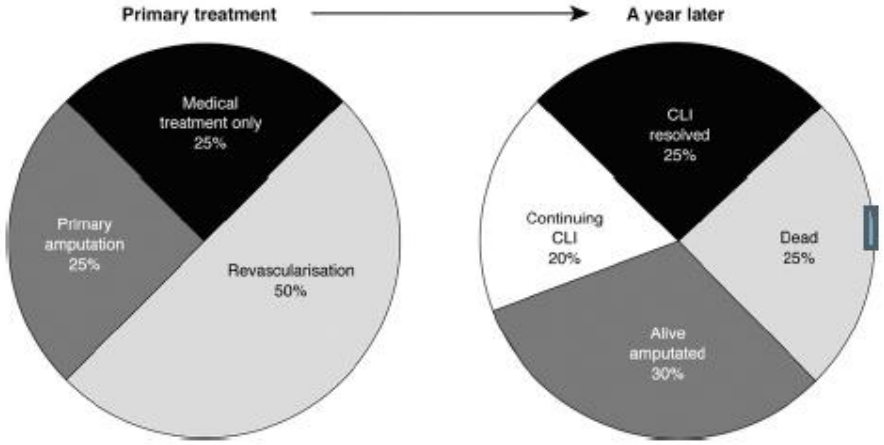


Fig. A5. Fate of the patients presenting with chronic critical leg ischemia. CLI – critical limb ischemia.

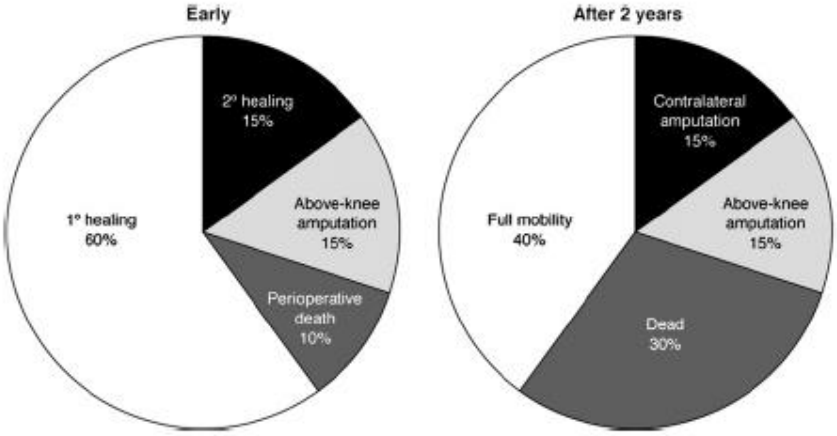


Fig. A6. Fate of the patient with below-knee amputation.

ANNEX 6: MEDIATORS OF INFLAMMATORY PAIN (40)

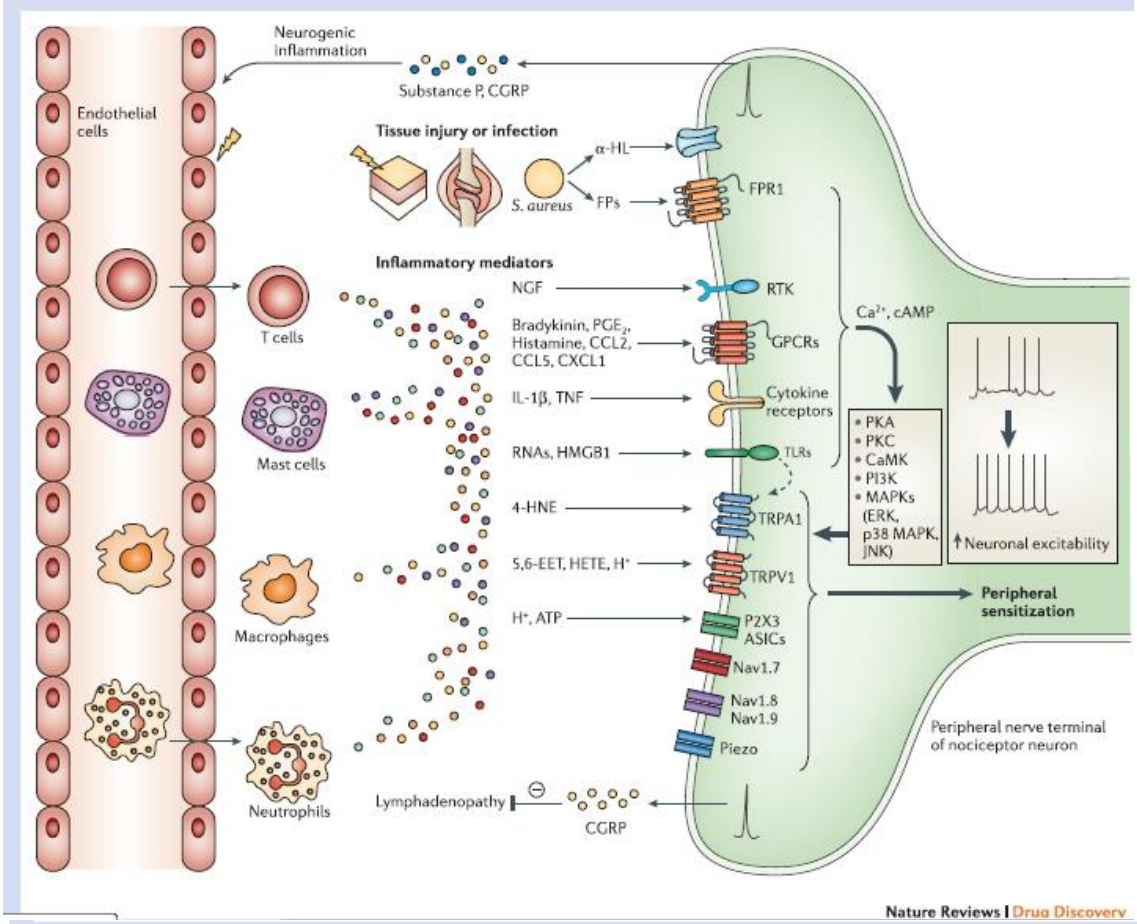


Fig 1 Mediators of inflammatory pain. Local inflammatory cells and infiltrating cells release mediators including pro-inflammatory cytokines [tumour necrosis factor (TNF; formerly known as TNFα), bradykinin, prostaglandins, interleukin-1β (IL-1β), and interleukin-6 (IL-6)], H^+, ATP, nerve growth factors (NGFs), and pro-inflammatory chemokines [CC-chemokine ligand 2 (CCL2), CXC-chemokine ligand 1 (CXCL1) and), CXC-chemokine ligand 5 (CXCL5)]. Receptors for these inflammatory mediators, including G-protein-coupled receptors (GPCRs), ionotropic receptors, and tyrosine kinase receptors, are expressed on nociceptive neurones. Stimulation causes generation of second messengers, such as cyclic AMP and Ca^{2+}, and downstream activation of kinases [protein kinase A (PKA), protein kinase C (PKC), extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase (PI3K), calcium/calmodulin-dependent protein kinase (CaMK), and the mitogen-activated protein kinases (MAPKs), p38 MAPK, and JUN N-terminal kinase (JNK)]. Activation of these kinases causes peripheral sensitization by modulating transduction molecules (e.g. transient receptor potential cation channel subfamily A member 1 (TRPA1) and TRPV1) and conduction (voltage-gated sodium channels Nav1.7–1.9). Activated nociceptors release substance P and calcitonin gene-related peptide (CGRP), involved in the generation of neurogenic inflammation and (CGRP) regulation of lymphadenopathy. Bacterial infection with *Staphylococcus aureus* induces neuronal hyperexcitability by releasing bacterial N-formylated peptides (FPs) and the formation of the pore-forming toxin α-haemolysin (α-HL). The bacteria can also directly activate nociceptors. 4-HNE, 4-hydroxynonenal; 5,6-EET, 5,6- epoxyeicosatrienoic acid; ASIC, acid-sensing ion channel; FPR1, formyl peptide receptor 1; HETE, 5-hydroxyeicosatetraenoic acid; HMGB1, high mobility group protein B1; P2X3, P2X purinergic receptor 3; PGE_2, prostaglandin E_2; RTK, receptor tyrosine kinase. Figure reproduced with permission from Macmillan Publishers Ltd.[41]

ANNEX 7: MEDIATORS OF NEUROPATHIC PAIN (41)

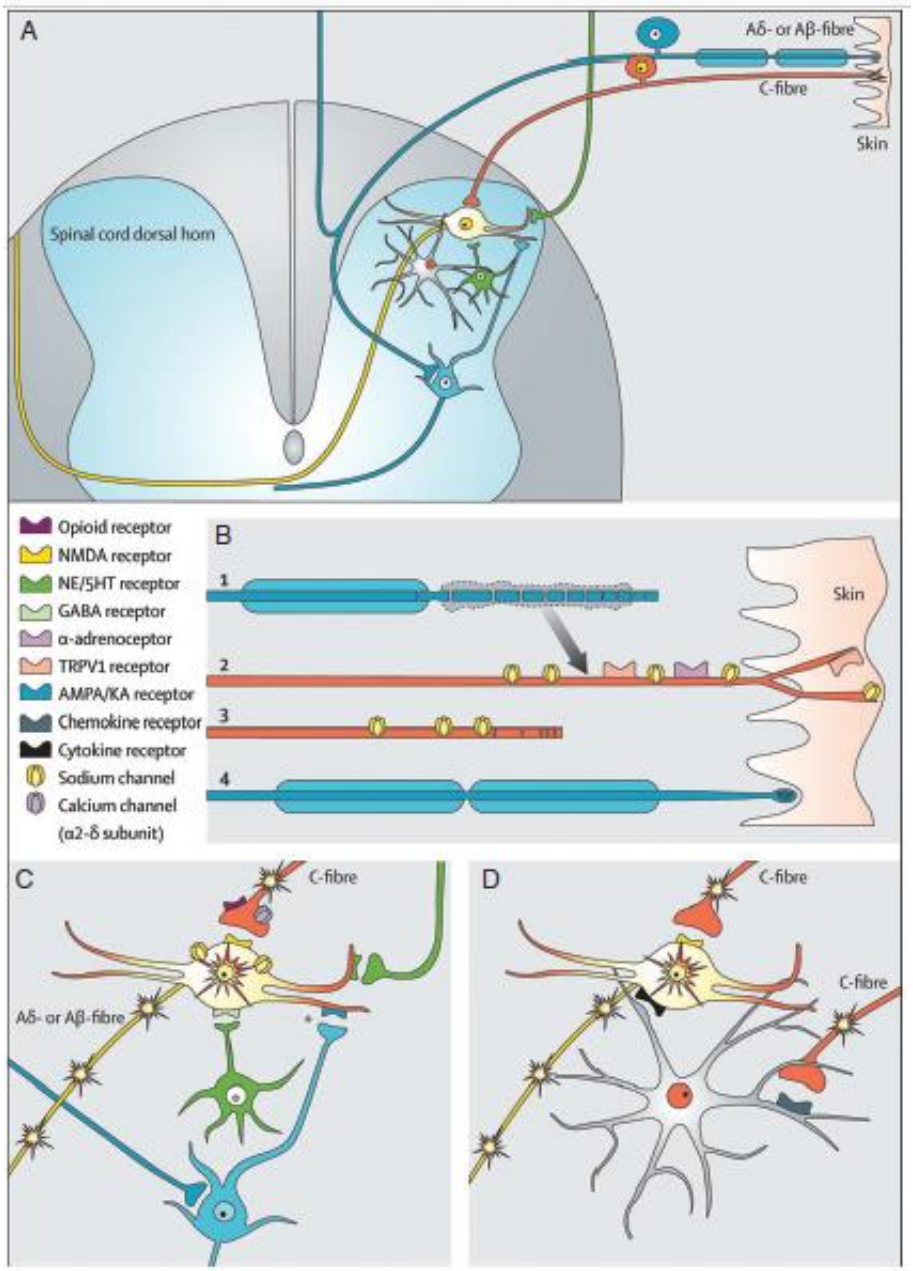
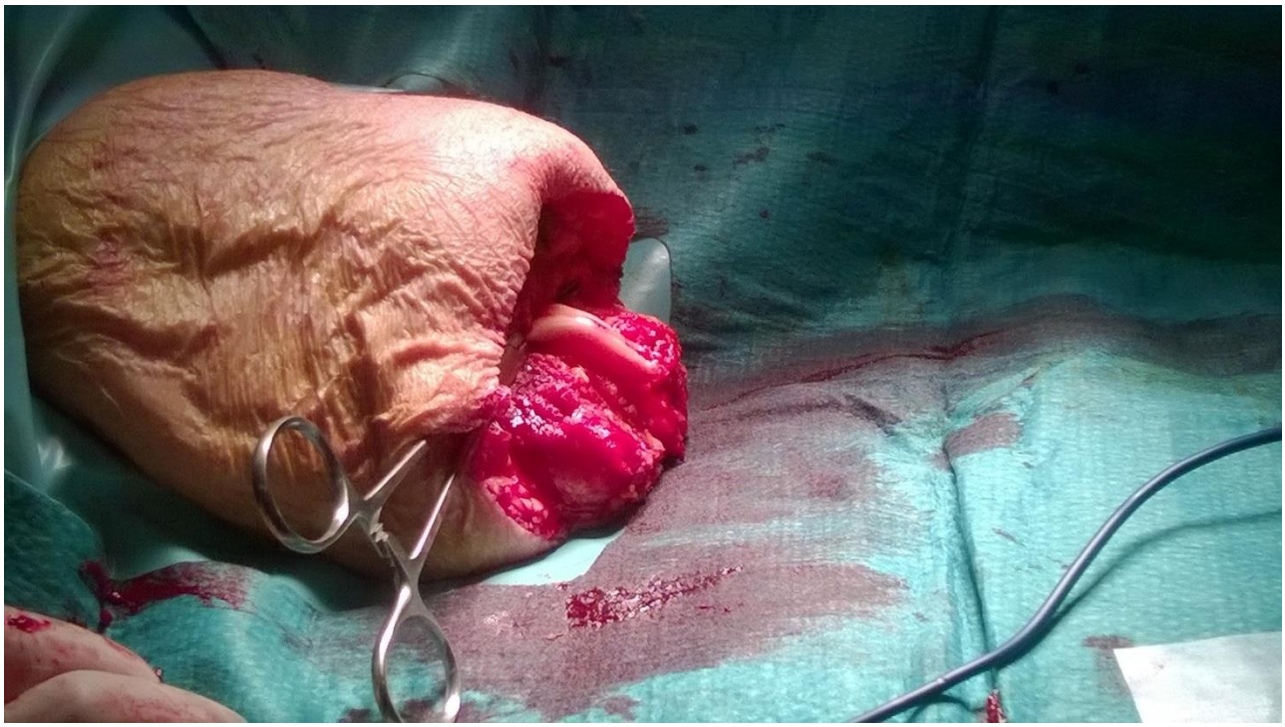


Fig 2 Mechanisms of neuropathic pain. (A) Primary afferent pathways connecting to the spinal cord dorsal horn. Nociceptive C fibres (red) terminate in upper laminae (yellow neurone), whereas non-nociceptive myelinated A fibres project to deeper laminae. Second-order neurones (WDR type) receive direct nociceptive input, synaptic input, and multisynaptic input from myelinated A fibres (non-noxious information; blue neurone system). Microglia (grey cell) facilitate synaptic transmission. GABAergic interneurones (green neurone) normally exert inhibitory synaptic input on the second-order neurone. Descending modulatory systems synapse at the second-order neurone (only the inhibitory projection; green descending terminal). (B) Peripheral changes underpinning peripheral sensitization at primary afferent neurones after damage. Note that some axons are damaged and degenerate (axons 1 and 3), whereas some remain intact and connected to the peripheral end organ (skin; axons 2 and 4). Expression of sodium channels is increased on damaged neurones (axon 3). Products such as nerve growth factor are released in the vicinity of spared fibres (arrow). These trigger the expression of channels and receptors (e.g. sodium channels, TRPV1 receptors, and adrenoreceptors) on uninjured fibres. (C) Spontaneous activity in C nociceptors induces spinal cord hyperexcitability (central sensitization of second-order nociceptive neurones; star in yellow neurone). These cause input from mechanoreceptive A fibres (blue neurone system; light touching and punctate stimuli) to be perceived as pain (dynamic and punctate mechanical allodynia; + indicates gating at synapse). Several presynaptic (opioid receptors and calcium channels) and postsynaptic molecular structures (glutamate receptors, AMPA/kainate receptors, sodium/5-HT receptors, GABA receptors, and sodium channels) are involved in central sensitization. Inhibitory interneurones and descending modulatory control systems (green neurons) are dysfunctional after nerve lesions. (D) Peripheral nerve injury activates spinal cord glial cells (grey cell) via chemokines, such as CCL2, acting on chemokine receptors. Activated microglia further enhance excitability in second-order neurones by releasing cytokines and growth factors (e.g. tumour necrosis factor and bone-derived nerve factor) and increasing glutamate concentrations. CCL2, chemokine (C-C motif) ligand 2; KA, kainate; NE, norepinephrine; TRPV1, transient receptor potential V1; WDR, wide dynamic range. Figure reproduced with permission from Elsevier.[23]

ANNEX 8: TRANSECTION OF SCIATIC NERVE DURING AMPUTATION SURGERY (in this case, AKA)



ANNEX 9: SHORT FORM OF MCGILL PAIN QUESTIONNAIRE

Short-Form McGill Pain Questionnaire

PATIENT'S NAME: _____ DATE: _____

	NONE	MILD	MODERATE	SEVERE
THROBBING	0) _____	1) _____	2) _____	3) _____
SHOOTING	0) _____	1) _____	2) _____	3) _____
STABBING	0) _____	1) _____	2) _____	3) _____
SHARP	0) _____	1) _____	2) _____	3) _____
CRAMPING	0) _____	1) _____	2) _____	3) _____
GNAWING	0) _____	1) _____	2) _____	3) _____
HOT/BURNING	0) _____	1) _____	2) _____	3) _____
ACHING	0) _____	1) _____	2) _____	3) _____
HEAVY	0) _____	1) _____	2) _____	3) _____
TENDER	0) _____	1) _____	2) _____	3) _____
SPLITTING	0) _____	1) _____	2) _____	3) _____
TIRING/EXHAUSTING	0) _____	1) _____	2) _____	3) _____
SICKENING	0) _____	1) _____	2) _____	3) _____
FEARFUL	0) _____	1) _____	2) _____	3) _____
PUNISHING/CRUEL	0) _____	1) _____	2) _____	3) _____

VAS NO PAIN ——— WORST POSSIBLE PAIN

PPI

0 NO PAIN _____
1 MILD _____
2 DISCOMFORTING _____
3 DISTRESSING _____
4 HORRIBLE _____
5 EXCRUCIATING _____

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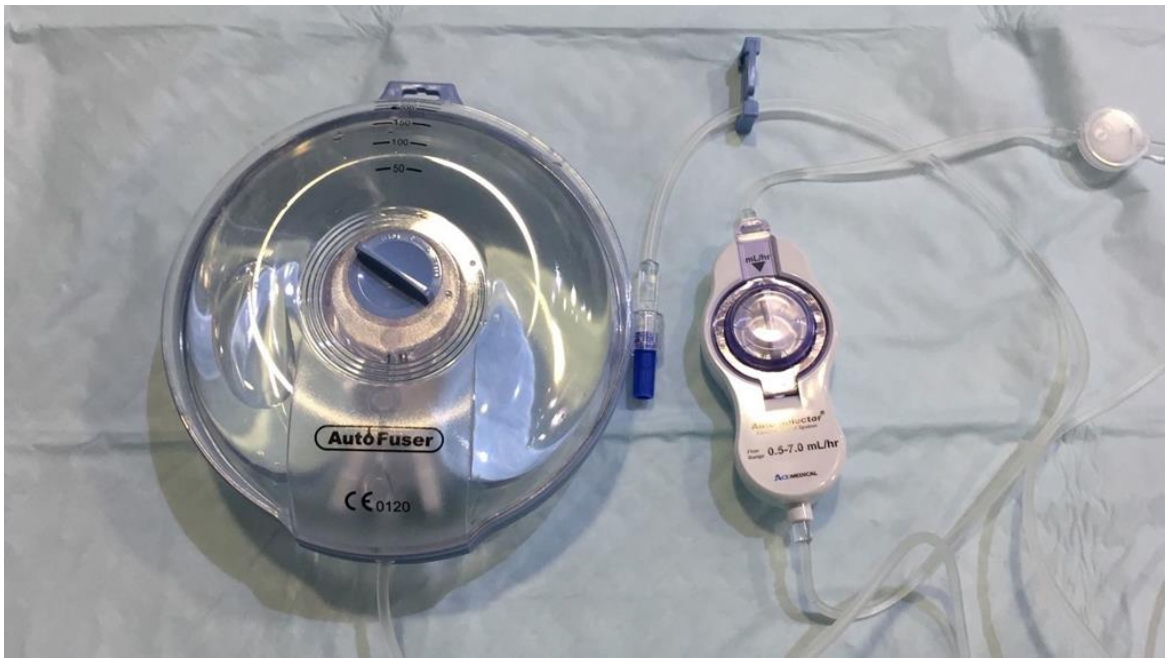
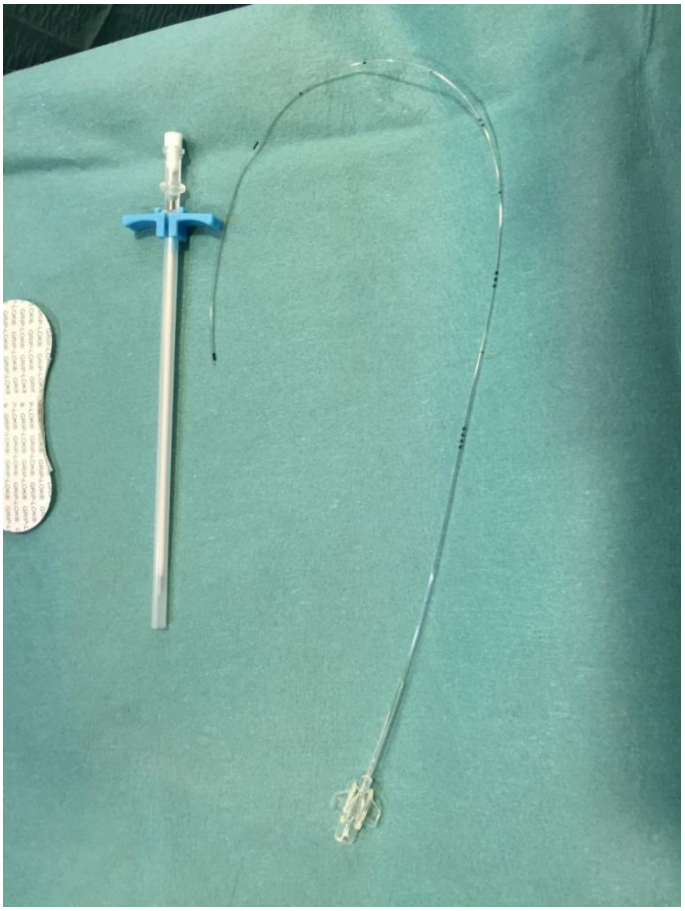
Descriptors 1-11 represent the sensory dimension of pain experience and 12-15 represent the affective dimension. Each descriptor is ranked on an intensity scale of 0= none, 1= mild, 2= moderate, 3=severe. The Present Pain Intensity (PPI) of the standard long-form McGill Pain Questionnaire and the VAS are also included to provide overall intensity scores.

ANNEX 10: ASA SCALE (CLASSIFICATION REGARDING ANESTHETIC RISK)

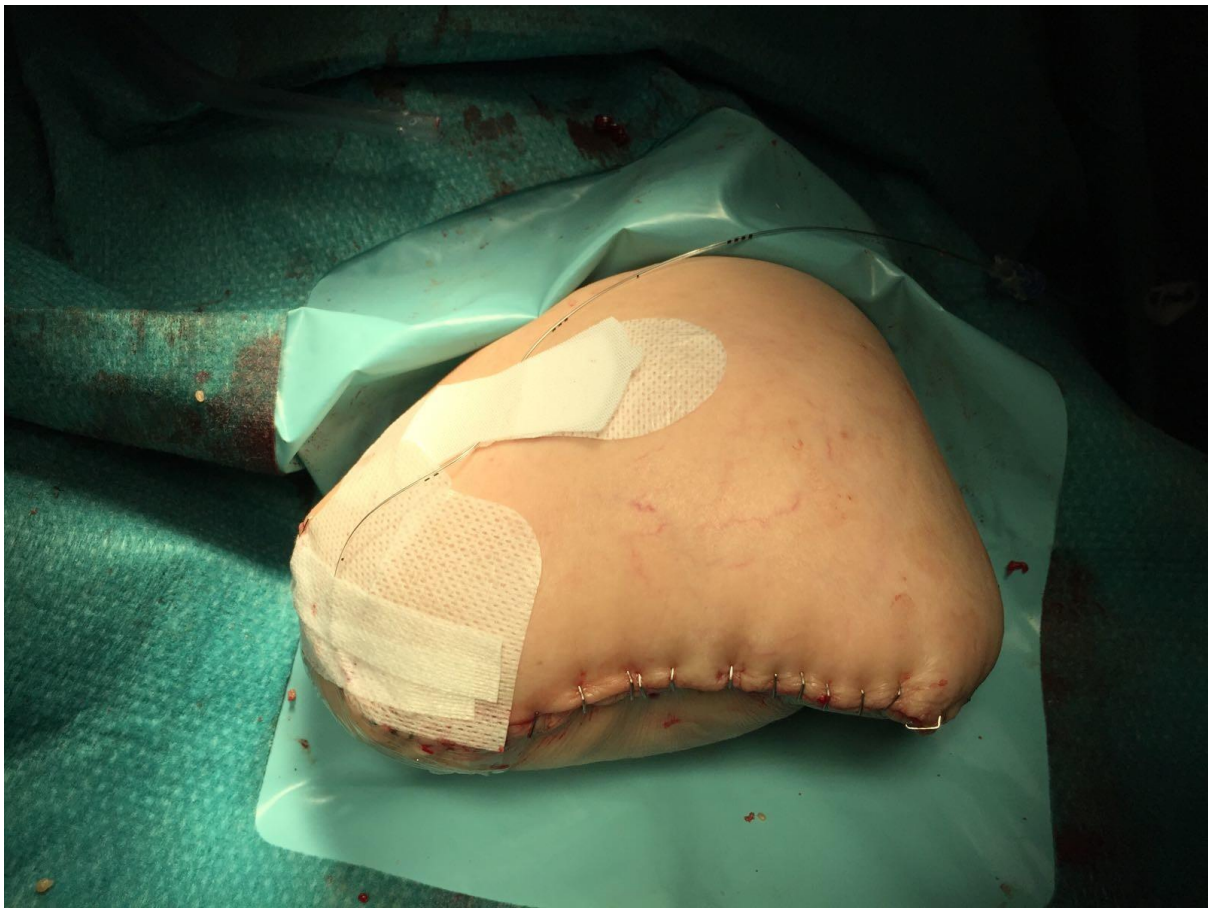
ASA PS Classification	Definition	Examples, including, but not limited to:
ASA I	A normal healthy patient	Healthy, non-smoking, no or minimal alcohol use
ASA II	A patient with mild systemic disease	Mild diseases only without substantive functional limitations. Examples include (but not limited to): current smoker, social alcohol drinker, pregnancy, obesity (30 < BMI < 40), well-controlled DM/HTN, mild lung disease
ASA III	A patient with severe systemic disease	Substantive functional limitations; One or more moderate to severe diseases. Examples include (but not limited to): poorly controlled DM or HTN, COPD, morbid obesity (BMI ≥40), active hepatitis, alcohol dependence or abuse, implanted pacemaker, moderate reduction of ejection fraction, ESRD undergoing regularly scheduled dialysis, premature infant PCA < 60 weeks, history (>3 months) of MI, CVA, TIA, or CAD/stents.
ASA IV	A patient with severe systemic disease that is a constant threat to life	Examples include (but not limited to): recent (< 3 months) MI, CVA, TIA, or CAD/stents, ongoing cardiac ischemia or severe valve dysfunction, severe reduction of ejection fraction, sepsis, DIC, ARD or ESRD not undergoing regularly scheduled dialysis
ASA V	A moribund patient who is not expected to survive without the operation	Examples include (but not limited to): ruptured abdominal/thoracic aneurysm, massive trauma, intracranial bleed with mass effect, ischemic bowel in the face of significant cardiac pathology or multiple organ/system dysfunction
ASA VI	A declared brain-dead patient whose organs are being removed for donor purposes	

ANNEX 11: ELASTOMERIC BOMB AND CATHETER





ANNEX 12: CATHETER PLACEMENT AFTER LOWER LIMB AMPUTATION



ANNEX 13: HOJA DE INFORMACIÓN AL PACIENTE

Estudio de dolor postoperatorio y miembro fantasma en pacientes amputados de extremidad inferior por enfermedad arterial periférica. Eficacia de infusión continua en 72 h postoperatorias a nivel del muñón con bupivacaína.

Nos gustaría que considerara su participación en este estudio prospectivo. Por favor, lea atentamente la siguiente información y tómese el tiempo necesario para decidir si desea o no participar. Gracias de antemano por la lectura de este documento.

El dolor durante el postoperatorio de la amputación de la extremidad inferior es una causa de "disconfort" en este periodo que puede alargar su estancia en el hospital, aumentar la necesidad de fármacos opiáceos (con los efectos adversos que estos conllevan) e incluso influenciar la aparición de fenómenos más complejos como el dolor del miembro fantasma, y el dolor residual del muñón. Ambos dificultarán su recuperación a largo plazo debido a su difícil e insatisfactorio manejo y a que afectarán a su calidad de vida, tanto a nivel funcional, en la colocación de prótesis para la deambulación, como a nivel psicosocial.

A lo largo de los años se han realizado una serie de estudios en pacientes sometidos a amputación de extremidad inferior a causa de estadios finales e irreversibles de enfermedad arterial periférica en los que se ha sugerido la eficacia y seguridad de la infusión local de anestésicos (como bupivacaína) a través de un catéter colocado durante la cirugía a nivel muñón.

Nuestro **objetivo** es confirmar que el tratamiento con bupivacaína en infusión continua durante las primeras 72 horas del postoperatorio es una técnica eficaz a la hora de disminuir el dolor inmediato a la amputación, disminuyendo así la influencia que éste ejerce sobre la aparición del dolor de miembro fantasma y dolor residual del muñón. Asimismo queremos confirmar también la seguridad de esta técnica, ya que al proporcionar un mejor control del dolor ayuda a disminuir la administración de otros fármacos, principalmente los opioides, con los efectos adversos que estos conllevan: náuseas, vómitos, sedación, delirium, prolongación de la estancia hospitalaria y aumento de la mortalidad.

Debe saber que para valorar el tratamiento durante el estudio habrá dos grupos de pacientes, uno de ellos recibirá bupivacaína a través del catéter localizado en el muñón y el otro grupo recibirá suero salino fisiológico. La distribución de los grupos se hará de forma totalmente aleatoria. Ambos grupos dispondrán de un dispositivo conocido como PCA (Analgesia Controlada por el Paciente) con

el cual usted podrá administrarse morfina, si la necesita para controlar el dolor y además se le prescribirán paracetamol y dexketoprofeno, por lo que sus necesidades analgésicas estarán cubiertas en todo momento. Asimismo tendrá que puntuar su dolor en una escala llamada Escala Visual Analógica a intervalos regulares de tiempo durante estos 3 días postoperatorios. Finalmente, a los 3 y 6 meses de la cirugía recibirá una llamada por teléfono para rellenar un cuestionario con el fin de informar de hallazgos relacionados con la aparición de dolor de miembro fantasma. En todo momento dispondrá de un teléfono de contacto o busca del médico responsable para que pueda comunicar cualquier incidente.

Su participación es **voluntaria** y podrá abandonar el estudio sin necesidad de dar explicaciones. La información obtenida será utilizada con fines científicos. Todos los datos serán considerados confidenciales y sólo su médico conocerá su identidad.
Todos los datos recogidos en este proyecto serán recogidos de forma anónima, siguiendo estrictamente las leyes y normas de protección de datos en vigor (Ley 41/2002 de 14 de Noviembre; Ley 15/1999 de 13 de Diciembre).

Con el fin de proteger la confidencialidad de la información personal de los participantes se han tomado las siguientes medidas:

- Todos los datos que puedan identificar al participante se mantendrán separados del resto de la información recogida en los diferentes cuestionarios del estudio.
- Cada caso del estudio contará con un número de identificación que será el que figure en las bases de datos. El análisis de la información se hará siempre de forma agregada y nunca individual.
- Todos los investigadores implicados en el proyecto se comprometen a cumplir las normas necesarias para preservar la confidencialidad de la información facilitada por los participantes.
- Los datos personales se desvincularán permanentemente de los datos clínicos y de los datos de las evaluaciones con el fin de proteger la identidad de los participantes (método de anonimización de los datos).
- La base de datos del proyecto estará protegida electrónicamente con códigos que limiten el acceso únicamente a las personas designadas (investigadores del proyecto).

ANNEX 14: FORMULARIO DE CONSENTIMIENTO INFORMADO

Estudio de dolor postoperatorio y miembro fantasma en pacientes amputados de extremidad inferior por enfermedad arterial periférica. Eficacia de infusión continua en 72 h postoperatorias a nivel del muñón con bupivacaína.

Nombre del médico: Dr……..

Fecha: …… Firma: ……..

1. Confirmo que he leído la hoja informativa del estudio con fecha……/…../…… Se me ha entregado una copia firmada y fechada de este formulario de consentimiento y la hoja de información para el paciente. Se me ha concedido tiempo y la oportunidad de formular preguntas sobre el estudio y todas ellas han quedado contestadas.
2. Comprendo que mi participación es voluntaria y que soy libre de retirar el consentimiento en cualquier momento, sin necesidad de ofrecer ninguna razón y sin que ello afecte a mis derechos legales ni a mi tratamiento médico en el futuro.
3. Soy consciente de que, al participar en el estudio, se recogerán y procesarán datos personales confidenciales. Se me ha informado con detalle de los motivos por los que se recogen y procesan estos datos y de quién tendrá acceso a ellos y se me ha explicado que tengo derecho a acceder a esta información y rectificarla.
4. Comprendo que mis ficheros médicos podrán ser revisados por las personas involucradas en el estudio si ello es importante para la investigación. Concedo permiso a estas personas para acceder a estos ficheros.
5. Accedo a participar en el estudio mencionado y autorizo la recogida y procesamiento de mis datos personales.

Nombre del paciente Fecha Firma