ADVANCES IN HEALTH AND DISEASE

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Lowell T. Duncan
Editor

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ADVANCES IN HEALTH AND DISEASE

VOLUME 9

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VOLUME 9

LOWELL T. DUNCAN
EDITOR

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PREFACE

Ascites is a pathological accumulation of fluid in the peritoneal cavity. Clinical manifestations largely depend on the volume accumulated, so ascites may present as a radiological finding or as an evident increase of abdominal perimeter. Advances in Health and Disease. Volume 9 opens with a discussion of the indications for paracentesis, an invasive procedure consisting of the extraction of ascitic fluid through percutaneous puncture of the abdominal wall, as well as techniques for diagnostic and therapeutic purposes, prophylactic considerations in order to minimize complications and alternative strategies.

The authors go on to discuss the knowledge provided by scientific research regarding tendon injury, summarizing how to approach injuries of the tendon in a concrete and schematic matter.

The goal of the following study is to evaluate pain relief in patients with chronic low back pain and failed back surgery syndrome after a single-shot intrathecal administration of midazolam. The analgesic effect was determined using a patient questionnaire during subsequent visits to the pain therapy service.

An additional study is presented in which 38 patients were treated with open reduction internal fixation via a dorsal approach. Clinical assessments included range of motion measurements at the wrist, grip strength, the Quick

Disability of the Arm, Shoulder, and Hand score, and the Gartland and Werley score.

Also in this compilation, the authors investigate combinations of methods and resources for a quicker and complete functional recovery. This research included 106 patients divided into two groups (with and without labor activities). 51 patients had a traumatized dominant hand and 47 had a complication of M. Zudeck. The analysis of the results obtained allows for the confirmation of the medical and social effectiveness of the complex Physiotherapeutic Rehabilitation Program.

Meckel's diverticulum is the most common congenital anomaly of the gastrointestinal tract, affecting approximately 2-4% of the population. In the case of bleeding, it is proposed that tagged RBC scintigraphy or angiography could be utilized to determine the source of bleeding and possibly direct the clinician towards the diagnosis of Meckel's.

The following chapter outlines advances that have been made within the growing field of zebrafish as a model for human diseases and highlights their advantages for drug discovery.

Furthermore, the zebrafish in vivo model presents many advantages when used in biotechnology and for food science and technology purposes. A large clutch size, transparent embryos, low-cost, fast growth and easy handling are the main features of this model. Recently, the zebrafish model has been used to study vertebrate development and for modeling human diseases and processes such as oxidative stress, liver damage, inflammation and evaluation of inhibition of lipid peroxidation.

The closing experiment was conducted in twenty-six pediatric patients who were given initial doses of continuous infusion of 200 μg/kg/h of midazolam, either from the innovator brand or from the generic brand. The children were monitored for plasma concentrations determined by high performance liquid Chromatography and the sedative effect was measured using a BIS monitor.

Chapter 1 - Ascites is a pathological accumulation of fluid in the peritoneal cavity. Clinical manifestations largely depend on the volume accumulated, so ascites may present as a radiological finding or as an evident increase of abdominal perimeter. Although the most frequent cause of

ascites is decompensated cirrhosis, there are a wide variety of diseases that may present with this symptom, such as cardiologic or renal diseases, malignant and infectious conditions, acute pancreatitis, venous or lymphatic drainage disorders, or malnutrition. Therefore, it is always important to elucidate its origin. It is usually associated with a poor prognosis, which largely depends on the underlying causes and treatment alternatives. Analysis of ascitic fluid is fundamental in the diagnostic process. Other relevant information can be provided through anamnesis together with radiological and laboratory tests. In selected patients, a systemic and splanchnic hemodynamic study, as well as a liver biopsy may be needed. Paracentesis is an invasive procedure consisting of the extraction of ascitic fluid through percutaneous puncture of the abdominal wall. Techniques vary depending on the need: either to obtain ascitic fluid for analysis (diagnostic technique) or to alleviate a massive ascites through large volume paracentesis (therapeutic approach). Usually the left lower quadrant of the abdominal wall is punctured, preferably with a needle or a trocar, whose length and gauge will depend on the anatomic features of the patient. Aspiration with the syringe is continuous until enough volume is achieved. In the case of therapeutic paracentesis, sterile closed drainage systems connected to the puncture device are required. Although it is an easy procedure, it is not free of complications. Most of them are usually mild, such as abdominal pain. However, some related and more serious adverse events are also possible such as hemoperitoneum, bowel perforation or severe circulatory dysfunction. There are some prophylactic tools aimed at minimizing the risk and severity of complications such as radiologic guided punctures, albumin infusion after large volume paracentesis or the correction of coagulation disorders prior to the procedure. Despite these risks, the clear advantages entirely justify the performance thereof. Nowadays, it remains an essential and irreplaceable tool, particularly from a diagnostic point of view. From a therapeutic perspective, different techniques have been developed which aim to replace large volume paracentesis, mainly in the refractory ascites secondary to liver cirrhosis setting. Indications for paracentesis, techniques for diagnostic and therapeutic purposes, prophylactic

considerations in order to minimize complications and alternative strategies for large volume paracentesis will be discussed throughout this chapter.

Chapter 2 - To this day, the knowledge provided by scientific research regarding tendon injury is minuscule in comparison to what is left to understand about it. However, there is much more known now than there was just a short while ago. In the past two decades, researchers have givenan important turn regarding these injuries. This is why it is a shame that when most clinicians say "tendon," the only thing that they still relate it to, is with treatment through eccentric exercise in best case scenarios, if not with passive models of manual therapy. Advances in science should suppose changes in the authors' clinical practice, and this new information is starting to be heard in the community of physical therapists closer to current research, but it is not an extended knowledge, and even less, used regularly in the authors' daily practice with patients that suffer this type of problems. This is why in this chapter the authors will summarize what we should be doing today about injuries of the tendon and how to approach them in a concrete and schematic matter.

Chapter 3 - *Background.* The antinociceptive effect of intrathecal midazolam is based on its affecting spinal gamma-amino butyric acid receptors. *Objective.* To evaluate pain relief in patients with chronic low back pain and failed back surgery syndrome after a single-shot intrathecal administration of midazolam. *Design.* A prospective, open-label study. *Outcome Measures.* The analgesic effect was determined using a patient questionnaire during subsequent visits to the pain therapy service. The authors classified a pain reduction of 50% or more as a positive outcome with improvement in quality of life and functional condition. *Results.* Between 1995 and 2017 the authors performed 748 administrations: 319 administrations in 63 male patients and 429 administrations in 85 female patients. The authors performed 93 administrations for chronic low back pain and 655 administrations for failed back surgery syndrome. The average age of the authors' patients was 51.8 years (range 28 to 86). The dose administered ranged from 2 to 5 mg of midazolam. The analgesic effect lasted 9 weeks on average, ranging from 1 week to 3 years; median of 5 weeks. In 65% of patients the authors achieved pain relief lasting 4 weeks

or longer; in 11%, the administration provided no analgesic effect at all. The incidence of side effects (drowsiness, nausea, headache, or transient worsening of complaints) was rather low. *Conclusion.* Intrathecal midazolam is a useful supplement to standard analgesic therapy with opioids, non-opioids, or spinal steroids.

Chapter 4 - *Introduction.* Despite the frequency of distal radius fractures, a consensus has not been reached on the optimal approach to treatment. Nevertheless, the volar plate is not a panacea for all distal radius fractures. Lutsky and colleagues reported that certain pathologic fracture patterns are more appropriately stabilized using dorsal plate fixation, and these include: (1) dorsal shear fractures, (2) dorsal *die-punch* fractures, or fracture patterns in which an indirect reduction from the volar approach cannot be obtained, and (3) fractures with associated scapholunate ligament injury. *Methods.* According to Lutsky's indications, the authors treated 38 patients were treated with open reduction internal fixation via a dorsal approach. Clinical assessments included range of motion measurements at the wrist, grip strength, the Quick Disability of the Arm, Shoulder, and Hand score, and the Gartland and Werley score. *Results.* Except for wrist flexion, favorable results were obtained for both subjective and objective parameters. Complications were rare and not serious. The most common reason for choosing dorsal plating was irreducible dorsal *die-punch* fractures. *Conclusion.* The treatment of displaced intra-articular distal radius fractures with a dorsally placed interlocking plate system demonstrated similar clinical results as volar locking plate. Postoperative complications were not readily observed in the patients treated with a dorsal locking plate. Certain fracture patterns are more appropriately stabilized using a dorsal plate fixation.

Chapter 5 - *Introduction:* The interest in rehabilitation of patients recovering after distal radius fracture is determined by the importance of the upper limp for performing all daily activities. The achievements of contemporary physiotherapy and rehabilitation medicine are significant and the scientific research in this direction is realized in different aspects. The main focus is on investigating combinations of methods and resources for a quicker and complete functional recovery. *Material and methods:* The authors' research included 106 patients divided into two groups (with and

without labor activities) – 51 patients had a traumatized dominant hand and 47 had a complication of M. Zudeck. For over sixty years the Clinic of Physical and Rehabilitation Medicine at the University Hospital in Pleven (Bulgaria) has been applying a complex physiotherapy and rehabilitation program. It includes: *Sub water gymnastics* – local bath tub with water temperature 34-36°C (in the area of wrist joint and forearm); *Kinesitherapy*; *Labor activities* (occupational therapy); *Impulse magnetic field* (15-20 min, 2 A, 1-100 Hz); *Interference currents* (5 min, 90-100 Hz; 10 min, 1-100 Hz); conducted daily for 10 days. The results are analyzed immediately after removal of the gypsum immobilization and at the end of the rehabilitation process. The authors have designed a special test to research and report *Comprehensive Functional Status* of patiens after trauma and diseases of the upper limb, including contractions in radioulnar joints, wrist joint, finger and thumb joints. The test contains five sections: (1) assessment of pain; (2) volume of movement of radioulnar joints, wrist joint, fingers and thumb; (3) MMT (Manual Muscle Testing) of pronation and supination in the radioulnar joints, flexion and extension in wrist joint and the muscles of the fingers and thumb; (4) tests of the grips; (5) Daily Life Activities (DLA) with separate parts for toilet and personal hygiene, dressing and shoes, preparing food and meals, various household activities. The maximum number of 100 points is an "Excellent" functional recovery. *Results:* The results obtained before and after rehabilitation, with damage to dominant and non-dominant upper limbs (with or without complication of M. Zudeck) show "Very good" and "Excellent" functional recovery of patients rehabilitated with inclusion of occupational activities, and "Good" recovery of those rehabilitated without it. In the case of complications of M. Zudek, neuritis of the n. median and shoulder-arm syndrome, the patients were treated with a complex rehabilitation program including labor activities up to 0,7 months (21 days), and in non-occupational patients – 1,1 month. The analysis of the results obtained gives us grounds to confirm, with high statistical significance, the medical and social effectiveness of the proposed complex Physiotherapeutic Rehabilitation Program including occupational activities in the treatment of patients with distal radius fracture. *Conclusion:* Improving the patient's quality of life requires the complete functional

recovery of the arm, which leads to the restoration of the patient's overall personality, his psyche, working capacity, social and economic status.

Chapter 6 - Meckel's diverticulum (MD) is the most common congenital anomaly of the gastrointestinal tract, affecting approximately 2-4% of the population. It is the result of an incompletely obliterated vitelline or omphalomesenteric duct around weeks 5-8 of gestation. Approximately 60% of Meckel's diverticula contain heterotopic mucosa, most commonly acid-secreting gastric mucosa. The lifetime incidence rate of complications from a Meckel's has been cited to be 4-6%. Common clinical presentations constituting symptomatic Meckel's are intussusception, volvulus, internal hernia, adhesions, Littre hernia, gastrointestinal bleeding (the most common presentations leading to the discovery of a Meckel's), diverticulitis, and perforation. Most Meckel's diverticula are asymptomatic and are discovered incidentally during imaging, endoscopy, or at the time of a surgical procedure. Technetium-99m pertechnetate scintigraphy (Meckel's Scan) has been utilized to attempt localization of the diverticulum. However reported sensitivities are as low as 60% but specificity is as high as 90-98%. Capsule endoscopy (CE) has come in vogue recently but is beset with deterring complications including risk of delayed passage or obstruction requiring surgical retrieval of the device. Other commonly used diagnostic modalities include plain abdominal radiographs, abdominal ultrasound, CT and MRI. Magnetic resonance enterography (MRE) has risen in popularity, particularly in bleeding patients, with a sensitivity in the range of 70% for localizing the site of origin of bleeding. For symptomatic patients, many advocate going to the operating room for exploration. Common indications for diagnostic laparoscopy or laparotomy are recurrent GI bleeds, chronic abdominal pain or persistent leukocytosis of unclear etiology, idiopathic ileus or persistent bowel obstructions, and repeated intussusceptions. In the case of bleeding, tagged RBC scintigraphy or angiography could also be utilized to determine the source of bleeding and possibly direct the clinician towards Meckel's. In brief, diagnosing Meckel's diverticulum can be very challenging, especially as the majority are asymptomatic, and those that are symptomatic can masquerade as other clinical entities such as appendicitis or bowel obstruction. Radiologic and nuclear medicine studies can be of use,

however if clinical suspicion is great enough, diagnostic laparoscopy will likely yield the diagnosis in a more direct fashion.

Chapter 7 - The development and process of diseases in zebrafish (*Danio rerio*) is strikingly similar to those of humans, allowing human diseases to be easily modeled in zebrafish by induction of chemical mutagens, transplantation or by genetic manipulations. Therefore, zebrafish have been gaining popularity as one of the favored animal model systems for studying human disorders and developing novel strategies against them. This chapter outlines advances that have been made within the growing field of zebrafish as a model for human diseases and highlights their advantages for drug discovery.

Chapter 8 - The zebrafish *in vivo* model presents many advantages when used in biotechnology and for food science and technology purposes. A large clutch size, transparent embryos, low-cost, fast growth and easy handling are the main features of this model. Recently, the zebrafish (*Danio rerio*) model, has been used to study vertebrate development and for modeling human diseases and processes such as oxidative stress, liver damage, inflammation and evaluation of inhibition of lipid peroxidation. Free radicals, such as superoxide (O_2), peroxyl (ROO), alkoxyl (RO), hydroxyl (HO), and nitric oxide (NO) play an important role in live organisms. Excessive free radicals induce various harmful effects in the human body such as cancer, liver injury, skin damage and aging. It is known that oxidative stress is caused by increasing the reactive oxygen species (ROS) which cause an imbalance with natural antioxidants that influences cell death and lipid peroxidation. Synthetic antioxidants, including butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have been used for food industrial purposes. However, recent research has focused on the extraction/identification of natural antioxidants from animal and vegetable sources. Recently, the zebrafish *in vivo* model has been used to evaluate the inhibition of lipid peroxidation using hydrogen peroxide and ethanol induced lipid peroxidation in quinoa, amaranth, red bean, milk proteins, walnut proteins, lysozyme and lysozyme peptides. For example, amaranth and quinoa have shown antioxidant activity and inhibition of lipid peroxidation in the zebrafish model. Proteins, hydrolysates

and peptides from hen egg white lysozyme presented antioxidant activity and inhibition of lipid peroxidation in zebrafish larvae. Gastrointestinal digests from *Chenopodium quinoa* Willd and *Amaranthus caudatus* L. have reported lipid peroxidation inhibition in the zebrafish larvae model. Casein and whey protein hydrolysates from cow inhibited lipid peroxidation in the zebrafish larvae model. The zebrafish embryos model is an excellent model to evaluate the *in vivo* inhibition of the formation of ROS using a fluorescent method. Zebrafish embryos and larvae can be used to evaluate the cytotoxicity of the natural antioxidants. Zebrafish embryos and larvae have been used as an anti-inflammatory model using LPS as a pro-inflammatory inductor.

Chapter 9 - Midazolam (MDZ) is used for sedation in Pediatric Intensive Care Units (PICU) prior to any clinical procedure. However, management of sedation requires the assessment of its level using some clinical scoring tools. In Pediatric Intensive Care Units, the most common tools used for assessing sedation levels are the modified Ramsay and COMFORT scales. Nevertheless, the point against these two scales is their subjectiveness. In contrast, the electroencephalographic (EEG) methods such as audio-evoked potentials and Bispectral Index (BIS) objectively analyze the degree of consciousness. Bispectral Index is a continuous measurement of the level of consciousness by analyzing the frequencies of EEG waves and from there estimates the degree of brain electrical activity and the sedation of the patient. The elimination of midazolam in PICU patients is low due to differences in age and disease status. Based on the possible existence of these variations, pharmacologists recommend that the best way to determine drug effect is to carry out therapeuric drug monitoring (TDM) to verify drug concentrations at different times. In view of this, TDM of midazolam was recommended, mainly to avoid the presence of adverse reactions. In the Pediatric Intensive Care Unit of the authors' hospital, it was found that with the brand of midazolam used, physicians have to apply higher infusion doses (6-folds higher) to reach the sedative status in children, with the risk of presenting adverse effects. This led us to conduct a study on the therapeutic efficacy of the two available brands of the drug – the innovator and the generic brands. The study was conducted in twenty-six pediatric patients

who were given initial doses of continuous infusion of 200 μg/kg/h, either from the innovator brand (Group A) or from the generic brand (Group B). The children were monitored for plasma concentrations determined by High Performance Liquid Chromatography (HPLC) and simultaneously, the sedative effect was measured using a BIS monitor. The results obtained were: *Group A*; Dose 284 (100-800) μg/kg/h, Plasma concentration (Cp) 725.3 (203.03 - 4633.04) ng/mL, BIS 72 (63 - 89), elimination half-time (t ½) 14.74 (2.1 - 56.7) h, and clearance (Cl) 3.04 (0.02-328.8) mL/h. *Group B*; Dose 500 (100-1200) μg/kg/h, Cp 4079.2 (694.4 - 7696.7) ng/ml, BIS 65.5 (42-82), t ½ 11.4 (1.1 - 57) h and Cl 1.29 (0.02 - 26.9) mL/h. Due to wide variation, the data are presented in medians and ranges. To achieve an adequate sedation, the results showed that twice the dose of the generic brand is required when compared with group A. The variation in the result may be explained by the commercial characteristics of the generic brand. This chapter analyzes the possible causes leading to variations in the sedative effect of midazolam in children.

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Chapter 1

ADVANCES IN HEALTH AND DISEASE: PARACENTESIS IN MEDICINE

Ana Clemente[1,2], Mario Romero[1,2,3], Diego Rincón[1,2,3,4], Jesus Millan[4,5], Rafael Bañares[1,2,3,4] and Rita Garcia Martinez[2,3,5,6,*]

[1]Hepatology Unit, Department of Digestive Diseases,
Hospital General Universitario Gregorio Marañón, Madrid, Spain
[2]Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain
[3]Centro de Investigación Biomédica en Red de Enfermedades
Hepáticas y Digestivas (CIBERehd), Madrid, Spain
[4]Universidad Complutense Madrid, Madrid, Spain
[5]Department of Internal Medicine,
Hospital General Universitario Gregorio Marañón, Madrid, Spain
[6]Facultad Medicina, Universidad San Pablo CEU, Madrid, Spain

[*] Corresponding Author Email: rita.garcia.martinez@gmail.com.

ABSTRACT

Ascites is a pathological accumulation of fluid in the peritoneal cavity. Clinical manifestations largely depend on the volume accumulated, so ascites may present as a radiological finding or as an evident increase of abdominal perimeter. Although the most frequent cause of ascites is decompensated cirrhosis, there are a wide variety of diseases that may present with this symptom, such as cardiologic or renal diseases, malignant and infectious conditions, acute pancreatitis, venous or lymphatic drainage disorders, or malnutrition. Therefore, it is always important to elucidate its origin. It is usually associated with a poor prognosis, which largely depends on the underlying causes and treatment alternatives. Analysis of ascitic fluid is fundamental in the diagnostic process. Other relevant information can be provided through anamnesis together with radiological and laboratory tests. In selected patients, a systemic and splanchnic hemodynamic study, as well as a liver biopsy may be needed.

Paracentesis is an invasive procedure consisting of the extraction of ascitic fluid through percutaneous puncture of the abdominal wall. Techniques vary depending on the need: either to obtain ascitic fluid for analysis (diagnostic technique) or to alleviate a massive ascites through large volume paracentesis (therapeutic approach). Usually the left lower quadrant of the abdominal wall is punctured, preferably with a needle or a trocar, whose length and gauge will depend on the anatomic features of the patient. Aspiration with the syringe is continuous until enough volume is achieved. In the case of therapeutic paracentesis, sterile closed drainage systems connected to the puncture device are required.

Although it is an easy procedure, it is not free of complications. Most of them are usually mild, such as abdominal pain. However, some related and more serious adverse events are also possible such as hemoperitoneum, bowel perforation or severe circulatory dysfunction. There are some prophylactic tools aimed at minimizing the risk and severity of complications such as radiologic guided punctures, albumin infusion after large volume paracentesis or the correction of coagulation disorders prior to the procedure.

Despite these risks, the clear advantages entirely justify the performance thereof. Nowadays, it remains an essential and irreplaceable tool, particularly from a diagnostic point of view. From a therapeutic perspective, different techniques have been developed which aim to replace large volume paracentesis, mainly in the refractory ascites secondary to liver cirrhosis setting.

Indications for paracentesis, techniques for diagnostic and therapeutic purposes, prophylactic considerations in order to minimize complications and alternative strategies for large volume paracentesis will be discussed throughout this chapter.

Keywords: ascites, portal hypertension, paracentesis, human, cirrhosis

LIST OF ABBREVIATIONS

ACLF	Acute-on-Chronic liver failure
ADA	Adenosine deaminase
AFP	α-fetoprotein
CA	Cancer antigen
CEA	Carcinoembryonic antigen
CLIF-C OF	Chronic Liver Failure Consortium Organ Failure
CLDQ	Chronic Liver Disease Questionnaire
FFP	Fresh frozen plasma
IEA	Inferior epigastric artery
INR	International Normalized Ratio
LDH	Lactate dehydrogenase
LVP	Large volume paracentesis
MHz	Megahertz
PCR	Polymerase chain reaction
PMN	Polymorphonuclear neutrophil
pPTL	Pooled platelets
PH	Portal hypertension
PPCD	Post-paracentesis circulatory dysfunction
RAAS	Renin-angiotensin-aldosterone system
SAAG	Serum-ascites albumin gradient
SBP	Spontaneous bacterial peritonitis
TIPS	Transjugular intrahepatic portosystemic shunt
TEG	Thrombelastography
TP	Total protein
US	Ultrasound
VAC	Vacuum-assisted closure

1. INTRODUCTION

The term paracentesis originally comes from the Greek word paraképntēsis ("*para": against. "kent": puncture*) and it refers to a puncture in the abdominal wall aimed at obtaining ascitic fluid for diagnostic purposes, as well as to alleviate tense ascites. Its performance is mandatory in the presence of ascites and it is an irreplaceable tool in a diagnostic work-up. It is an easy and safe procedure, although some complications, usually not severe, may occur. This chapter aims to review the initial diagnostic approach of ascites through diagnostic paracentesis, technical issues related to the procedure, potential related complications and prophylactic strategies to avoid them as well as alternative strategies for large volume paracentesis (LVP).

2. ASCITES

2.1. Concept and Diagnosis Approach

Ascites is a pathological accumulation of fluid in the peritoneal cavity. A small amount of free fluid (~50-75 ml) is considered a normal finding, since it is physiologically generated to lubricate the tissues of the abdominal wall and viscera [1-3]. Ascites manifestations largely depend on the amount of fluid accumulation, so it may present as a radiological finding or as an evident increase of abdominal perimeter. Ascites can be graded from 1 to 3 according to the amount of fluid in the abdominal cavity: Grade 1 or mild ascites is only detectable through ultrasound examination. Grade 2 or moderate ascites is defined by a moderate symmetrical distension of the abdomen while grade 3 or large ascites is defined by a marked abdominal distension [4]. Refractory ascites is the ascites that becomes untreatable with medical therapy due to the lack of response or the development of major side effects [5]. Clinically evident ascites must be explored by looking at the bulging flanks and the flank dullness to percussion, but it can only be

identified when there is approximately at least 500 ml of fluid present. These combined findings have shown a sensitivity of 75% and a specificity of 57%. Detection of a fluid wave or puddle sign is less reliable. Nevertheless, anatomic features of the patient, such as obesity, may complicate the physical examination. Radiologic techniques, especially an abdominal ultrasound, can easily demonstrate not only large ascites but also smaller amounts of fluid, and due to its non-invasive profile, it is the first-line imaging method used to confirm the presence of ascites. A CT-scan and magnetic resonance imaging (MRI) can also provide valuable information, however, both require the administration of contrast, which is expensive, and are usually less available and a CT-scan exposes the patient to x rays, which makes these techniques a second step approach in the diagnosis of ascites [2, 6].

2.2. Differential Diagnosis

Once ascites is confirmed, elucidating its origin is mandatory. Liver cirrhosis is the most common cause of ascites in adults in the Western world, which is responsible for about 80% of cases [4]. However ascites may also appear in a broad spectrum of diseases driven by different underlying pathophysiological mechanisms such as cardiologic or renal diseases, malignant and infectious conditions, acute pancreatitis, venous or lymphatic drainage disorders, or malnutrition [6]. Its prognosis largely depends on the primary disease and the treatment alternatives but it is usually associated with a poor outcome, particularly in cirrhosis.

Differential diagnosis requires an exhaustive clinical record focusing on the pattern of body weight gain, change in abdominal girth, presence of ankle edema as well as information about the patient's medical history, medication usage, lifestyle and risk factors. Besides the previously mentioned findings, a physical examination must be carried out with special attention paid to the signs of liver or heart disease as well as malignancy. Routine blood tests that include tumor markers are mandatory.

Table 1. Differential diagnosis of the main causes of ascites according to laboratory parameters

	Cirrhosis	Malignancy	Cardiologic	Nephogenic	Pancreatitis	Tuberculosis
Appearance	Clear straw or milky	Milky or bloody	Clear Straw or pale	Clear straw or pale	Turbid, cloudy or bloody	Clear straw or milky
Total protein (g/dl)	<2.5	≥2.5	<2.5	≥2.5	≥2.5	≥2.5
SAAG (g/dl)	≥ 1.1	< 1.1	≥ 1.1	< 1.1	< 1.1	< 1.1
Cell count (polymorphonuclear neutrophil; cells/mm$^{3)}$	<250	≥250	<250	<250	Variable	≥250 or normal
LDH	↓	↑	↓ or normal		↑ or normal	↑ or normal
Glucose	Normal	↓	Normal	Normal	↓	↓
Amylase	Normal	↑ or normal	Normal	Normal	↑	Normal
Bacterial culture	+ or – (If positive: "bacterascites")	-	-	-	-	+ or -
Tumor markers	↑ or normal	↑	Normal	Normal	↑ or normal	↑ or normal

In selected patients, the performance of a systemic and splanchnic hemodynamic study as well as a liver biopsy may be needed. In addition to all the standard routine methods, the performance of paracentesis and ascitic fluid extraction for further analysis is the most important step in the diagnostic work-up (Table 1).

2.2.1. Ascitic Fluid Analysis

2.2.1.1. Gross Appearance

Inspection of the appearance of ascitic fluid can provide useful preliminary information for its differential diagnosis [6-8]. Under normal conditions, ascites has a clear yellowish appearance.

Chylous or milky ascites is characterized by the presence of chylomicrons and a high concentration of triglycerides. Several diseases can lead to the development of chylous ascites such as traumatisms, lymphatic abnormalities, solid and hematologic malignancies, cirrhosis, infections, congenital defects, inflammatory processes and renal and cardiologic disorders [6, 7, 9].

Milky ascites must be distinguished from turbid, cloudy or, so-called, pseudochylous ascites, a finding which is usually related to a high neutrophils concentration secondary to bacterial infection, peritonitis, pancreatitis, or bowel perforation [6-10]. When a purulent fluid is obtained the ascitic fluid polymorphonuclear (PMN) leukocyte count is usually >50,000 cells/mm^3 [11].

Bloody ascites is often associated with malignancies, either as primary from abdominal viscera, for instance hepatocellular carcinoma, or secondary to carcinomatosis metastatic tumors. Non-malignant conditions can also lead to bloody ascites such as hemorrhagic pancreatitis or viscera perforation [6-8, 10, 11]. True bloody ascites shouldn´t be mistaken with blood originated in the peritoneal cavity due to a traumatic puncture. If a traumatic puncture is suspected, a second tap has to be performed on the opposite flank that will usually drain clear ascitic fluid elucidating that the initial tap was traumatic and not due to a pre-existing hemoperitoneum. Normally the fluid obtained from a traumatic tap will clot whereas non-traumatic bloody ascitic

fluid is usually homogeneously red and does not clot on removal. A tea-colored fluid can occasionally be seen in pancreatic ascites due to the red cell degradation by the pancreatic enzymes, exacerbated in hemorrhagic pancreatitis, leading to the appearance of a dark fluid named black ascites, which has also been reported in melanoma [11].

2.2.1.2. Non-Biochemical Tests

Cell Count

The cell count in ascitic fluid is the single most helpful test to perform on ascites [8, 11]. Since, as previously mentioned, cirrhosis is the most common cause of ascites, polymorphonuclear neutrophils counts should be performed on all patients with ascites that have been admitted to the hospital or are showing signs of clinical deterioration or suggestive of spontaneous bacterial peritonitis (SBP) [4, 6-8, 11-13]. Normal counts of PMNs in ascitic fluid is about 27-30% of the total white cell count and the cut-off value for a normal ascitic PMN count is <250 cells/mm^3 [4, 11].

SBP is a common complication in decompensated cirrhosis defined as a bacterial infection of ascitic fluid without any intra-abdominal surgically treatable source of infection [4, 12, 13] and it is diagnosed when the PMN count in ascitic fluid is >250cells/mm^3 (this is the cut-off with the highest sensitivity, although the greatest specificity is reached with a cut-off of 500 neutrophils/mm^3) [13]. Despite the fact that the gold standard for ascitic neutrophil count is manual microscopy, it was replaced in most places by automated counts based on flow cytometry due to the laborious and inter-observer variability inherent to the former one. Indeed, the flow cyto-metry technique has shown a sensitivity and specificity close to 100% for counting and differentiating cells [14]. Urine reagent strips have shown a poor accuracy for SBP diagnosis (using a threshold of 2+ for positivity of the reagent strip: sensitivity 45.3%, specificity 99.2%, positive predictive value 77.9%, and a negative predictive value of 96.9%) so it can no longer be recommended [4, 15]. The ascitic white cell count might occasionally be misinterpreted in the setting of a traumatic puncture. In this scenario, one PMN per 250 red cells can be attributed to contamination with blood, so the

PMN count has to be recalculated in order to avoid an erroneous diagnosis of SBP [12]. Since SBP is a serious complication of cirrhosis, an empirical antibiotic must be started immediately according to the local bacterial antibiotic resistance profile [4, 12, 13]. The so-called term bacterascites refers to the presence of ascitic fluid positive cultures but with a normal ascitic PMN count (<250/mm^3). As it may represent the first step in the development of SBP, management recommendations are similar to the latter, especially in those patients who show symptoms and signs of infection [4, 13].

In contrast to SBP, secondary bacterial peritonitis is a bacterial peritonitis due to the perforation or acute inflammation of intra-abdominal organs, abdominal infection or previous abdominal surgical procedures.

Differential diagnosis between both entities is mandatory since the management and prognosis differ, however it can occasionally be difficult, unless there is a previous known condition that helps to precisely elucidate the nature of the infection (e.g., surgery). In this regard, laboratory tests are useful. The proposed criteria for diagnosing secondary peritonitis are at least two of the following findings in the ascitic fluid: glucose levels of <50 mg/dl, protein concentration of >10 g/l, lactic dehydrogenase (LDH) concentration above the normal serum levels. Other criteria such as a very high ascitic neutrophil count, clinical evident abdominal symptoms or signs of intraabdominal surgical complications, isolation of multiple micro-organisms on ascitic culture or an inadequate response to therapy, support the diagnostic of secondary peritonitis [4, 12, 13, 16-18].

Besides PMN, other cell types routinely measured in ascitic fluid can also provide relevant information. Lymphocytes predominance has been reported in tuberculous peritonitis and peritoneal carcinomatosis. In addition, in chylous ascites due to a lymphatic leak, lymph-derived white cells may also be elevated. On the other hand, an elevated red cell count in ascitic fluid can be found in malignant diseases as well as in cardiac and chylous ascites secondary to a leakage of red cells from the congested liver or from a lymphatic tear [10, 11].

Cultures

Bacterial cultures are useful when they are positive, however they have a low sensitivity (42-65%) mainly due to a low median bacterial count in ascitic fluid and a delay in the time taken to inoculate the sample in the culture medium, which can lead to false-negative results [6, 8, 11]. Several studies have shown that bedside inoculation may improve culture sensitivity up to 70–90% [19]. Therefore, bedside inoculation of ascitic fluid in blood culture bottles, containing aerobic and anaerobic media (10-20 ml per bottle) is currently the recommended procedure. Additionally, ascitic fluid cultures should be carried out before antibiotic treatment is initiated [4, 12]. Mycobacterial cultures from ascitic fluid have an even lower sensitivity (20–35%) as well as acid-fast bacilli detection in the ascitic fluid (0–6%). Thus, when tuberculous peritonitis is suspected the value of these tests in the differential diagnosis of ascites is limited and the performance of bacterial DNA of *Mycobacterium tuberculosis* detection techniques are required instead [6, 9, 20].

Cytology

Performance of ascitic fluid cytology is indicated when malignancy related ascites is suspected or in those cases in which the underlying disease is not clarified [6]. Peritoneal carcinomatosis accounts for at least two thirds of patients with malignancy related ascites. In the remaining one-third the presence of massive liver metastasis, lymphoproliferative disorders or hepatocellular carcinoma justify the formation of ascites. Ascitic fluid cytology is extremely valuable in suspected cases of peritoneal carcinomatosis, where malignant cells shed into the ascitic fluid and can be detected with a specificity close to 97%. Nevertheless, the accuracy of cytology may differ depending on the underlying oncologic disease, especially in the absence of peritoneal carcinomatosis, such as hepatocellular carcinoma or liver metastasis, in which case, it hardly reaches 10% [10].

In order to maximize the results, when ascitic fluid is obtained for cytology, early submission to the laboratory in addition to collecting at least three samples of 50-1000 ml each from different paracentesis is advisable, since it seems to improve the sensitivity of the procedure [6, 8, 11].

Polymerase Chain Reaction (PCR)

Low sensitivity of ascitic fluid cultures represents a major drawback in the diagnosis of infectious related ascites and the early guided antimicrobial therapy thereof. However, recent advances in molecular techniques, particularly PCR development, provides a fast and reliable method for microbiological identification, especially for those microorganisms that are difficult to isolate, such as *Mycobacterium tuberculosis*, with 94% sensitivity and 88% specificity, as well as being a timesaving tool for the diagnostic decision making process [6, 7, 21].

Diagnostic Laparoscopy

Since it is an invasive procedure, its role is limited to those cases in which there is not a definitive aetiology, despite an in-depth diagnostic work-up. When indicated, diagnostic laparoscopy provides valuable information by allowing direct inspection of the abdominal cavity and the collection of samples for histological and microbiological studies. It seems to be particularly helpful in diagnosing peritoneal carcinomatosis, tuberculous peritonitis and other peritoneal diseases, for instance, mesothelioma and sclerosing peritonitis [6, 20].

2.2.1.3. Biochemical Tests

Total Protein

Total protein concentration in ascitic fluid has traditionally been used in the differential diagnosis of ascites according to a cut-off value of 2.5 g/dl to classify the ascites into transudate (<2.5 g/dl) or exudate (≥2.5 g/dl). Nevertheless, the scientific evidence on which this classification was based is not robust enough and, indeed, growing evidence disclosed a low accuracy of ascitic total protein to elucidate the cause of ascites frequently resulting in misclassifications. In consonance, ascites due to cirrhosis have been traditionally categorized as a transudate. However, several studies have reported concentrations higher than 2.5 g/dl in at least 12-24% of cirrhotic patients with ascites [22-24]. Moreover, concentrations lower than 2.5 g/dl can be found in patients with ascites secondary to malignancies in which

ascites had been traditionally categorized as an exudate [23-25]. Therefore, the total protein in ascites fluid is no longer recommended for classification and has been replaced by the serum-ascites albumin gradient (SAAG), a more sensitive and specific measurement to discriminate between ascites due to portal hypertension (PH) and ascites secondary to other pathophysiological mechanisms (Table 1) [26].

Despite its mentioned limitations, the total protein ascites fluid value is still a relevant parameter, particularly in cirrhosis related ascites, since cirrhotic patients with low ascitic fluid protein concentration (<10 g/l) have a high risk of developing SBP [27]. The beneficial effect of prophylactic antibiotics is that it decreases the risk of developing a first episode of SBP and hepatorenal syndrome as well as increasing the survival rate of this population, which has been proven by several randomized trials and represents a strong recommendation supported by a high level of evidence [4, 12]. As previously mentioned, the total protein in ascitic fluid is also useful to discriminate between spontaneous and secondary bacterial peritonitis.

Albumin and Albumin Gradient

Serum-ascites albumin gradient is a measurement obtained by subtracting the albumin concentration in ascitic fluid from the serum one. This parameter is based on a more pathophysiological approach than the total-protein-based exudate/transudate concept; it is thought that it reflects the oncotic pressure gradient (manly dependent on albumin) between the vascular bed and the ascitic fluid. Therefore, it helps to distinguish the ascites related and not related to portal hypertension [8]. Using the cut-off 1.1 g/dL, SAAG one can discriminate between the two categories: “high albumin gradient” (SAAG ≥ 1.1 g/dl) that indicates underlying portal hypertension or hepatic congestion and “low albumin gradient” (SAAG < 1.1 g/dl), which indicates that the ascites is secondary to a pathophysiological mechanism other than portal hypertension, such as malignancy, pancreatitis or infection [28].

SAAG seems to be able to reasonably differentiate ascites due to portal hypertension with an accuracy of up to 97% while the transudate/exudate

concept based on total protein level, barely classifies 55.6% of ascites properly [26]. As a consequence, SAAG replaced the ascitic total protein concentration as the first step for the differential diagnosis. Classification of ascites according to SAAG is shown in Table 2.

For maximum effectiveness, it is recommended to obtain blood and ascites samples at relatively the same time points since portal hypertension is a dynamic process. Although the SAAG allows for differentiating these two broad categories, it does not replace further evaluations. Once the cause of ascites has been established, it is not necessary to repeat the SAAG in subsequent paracentesis [1, 4, 11].

Table 2. Classification of ascites according to Serum-Ascites Albumin Gradient (SAAG)

SAAG ≥ 1.1 g/dl	SAAG < 1.1 g/dl
• Liver cirrhosis • Fulminant hepatic failure • Liver metastases • Budd-Chiari syndrome • Portal Vein Thrombosis • Veno-occlusive Disease • Myxedema • Cardiac Failure • Constrictive Pericarditis • Pulmonary Hypertension	• Malignancy • Peritoneal carcinomatosis • Nephropathy • Malnutrition • Protein-losing enteropathy • Tuberculous peritonitis • Pancreatic ascites • Serositis • Secondary peritonitis

Lactate Dehydrogenase

The significance of LDH concentration in ascitic fluid in the differential diagnosis of ascites has been previously explored. It is a nonspecific parameter, and its value fluctuates with infection and malignancy. Previous studies showed that the LDH concentration in ascitic fluid was significantly lower in patients with liver disease than in those with malignant disease. However, there is a significant overlap between both groups, and only high levels of LDH in ascitic fluid are associated with specific conditions such as malignancy, tuberculosis or pancreatic disease. Moreover, no LDH cut-offs

have been established in order to discriminate between hepatic disease and malignancy, and the latter cannot be excluded by a low LDH ascitic level [23].

When extrapolating Light´s criteria for pleural effusion [29] to discriminate between hepatic and non-hepatic ascites, LDH levels in conjunction with total protein might be more useful. The combination of two out of the three of these exudate criteria (ascites LDH 400 UI/L, ascites/serum LDH ratio 0.6 and ascites/serum TP ratio 0.5) suggests a non-hepatic cause of the ascites, whereas values below the cut-offs for the three parameters suggest that the ascites is related to hepatic disease [23]. Despite its limitations, its relevance seems to be limited to the diagnosis of secondary bacterial peritonitis, where the LDH ratio (ascites/serum LDH, normal value 0.4) can increase [30].

Glucose

Glucose diffuses easily trough membranes, thus its concentration in ascitic fluid tends to be similar to the serum glucose range. The presence of bacteria, white blood cells or cancer cells may decrease glucose levels in ascitic fluid due to its consumption. This finding seems to be especially useful to identify or to exclude secondary bacterial peritonitis when a large bacterial count is present, and active consumption occurs. Nevertheless, it is a marker with low diagnostic sensitivity and specificity, playing a limited role in the differential diagnosis of ascites [16, 30].

Amylase

The assessment of amylase in ascitic fluid should be carried out when there is a high suspicion of ascites related to a pancreatic disease. The ascitic fluid amylase concentration is usually within the normal range in non-pancreatic ascites but it increases in conditions in which amylase is released into the fluid surrounding the pancreatic gland such as acute or chronic pancreatitis, rupture of a pseudocyst or pancreatic duct disruption. Previous studies showed that when a leakage of pancreatic enzymes into the peritoneal cavity occurs, the ascitic amylase levels are often >1000 IU/L, with the ascitic/serum amylase ratio increasing up to 6.0 [31]. On the other

hand, a ratio of 0.4 is normal in non-pancreatic ascites. It has been suggested that the ascites to serum amylase ratio might have a prognostic role in the context of acute pancreatitis with clinically apparent ascites [32]. Nevertheless, high amylase levels in ascitic fluid may also be found in other pathological conditions such as small bowel perforation, ischemia, mesenteric thrombosis, and even in malignancies, therefore, it is not specific to pancreatic disease [16, 33].

Tumor Markers

Evidence regarding the usefulness of tumor markers in the diagnostic work-up of ascites, especially to distinguish malignant and non-malignant ascites is controversial. Tumor markers constitute a tool that is useful for cancer screening, diagnosis confirmation, prognosis, as well as treatment follow-up [34]. The most common tumor markers evaluated to discriminate between malignant and non-malignant ascites are α-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen (CA) 19-9, and CA125. Overall, many studies have showed that ascitic levels of these markers can be significantly higher in patients with different malignancies such as hepatocellular carcinoma, colorectal, pancreatic and ovarian cancer compared to non-malignant ascites [35].

However, elevation of such markers in ascitic fluid can be identified in the absence of malignancy, particularly in liver cirrhosis [34]. Likewise, concentrations above the normal range of CA-125, CA19-9 and CA15-3 have been reported in the peritoneal fluid of women with endometriosis and other benign pelvic disorders, especially CA-125 in ascites secondary to endometriosis [36]. Therefore, the increase of tumor markers in ascitic fluid in the absence of other findings that support the suspected diagnosis of malignancy must be interpreted with caution. Additionally, it has not clearly demonstrated an additional value compared to traditional serologic tumor markers but both share the same limitations regarding their diagnostic accuracy [37].

Nevertheless, recent studies suggest that the low diagnostic performance of these markers may improve when combined with cytology and when used in a panel combining different ascitic tumor markers [35, 38]. Therefore, its

routine analysis is not recommended and must be restricted to cases of suspected malignancy and inconclusive cytological findings in addition to further studies.

Urea and Creatinine

Urea and creatinine measurements in ascitic fluid can be useful in cases of urinary leakage into the peritoneal cavity. An increase in ascites/plasma creatinine ratios have been reported in cases of urinary ascites. Data regarding these parameters in differential diagnosis of ascites is scarce, since it relates to a rare complication and it is limited mainly to case reports [39, 40]. Nonetheless, these complementary measurements do not preclude the performance of a cystoscopy, imaging techniques or even a diagnostic laparoscopy when needed.

Adenosine Deaminase (ADA)

Several reports have shown that ascitic fluid adenosine deaminase activity (ADA) is a sensitive and specific marker for the early diagnosis of tuberculous ascites. A recent meta-analysis of four studies that included 264 patients confirmed a sensitivity and specificity of 100% and 97% respectively of ADA in ascitic fluid using cut-offs from 36 to 40 IU/L in the diagnosis of tuberculous ascites [41]. Conversely, some reports showed a lower diagnostic accuracy in areas of low prevalence and in patients with underlying cirrhosis [42].

Lipids

- *Triglycerides.* When milky-colored ascitic fluid is found, measurement of triglyceride levels is indicated. Chylous ascites is defined by an ascitic fluid with a triglyceride level of >200 mg/dl. Malignancy and cirrhosis account for over two thirds of all cases, while infections are responsible for the majority of cases in developing countries [43].
- *Cholesterol.* Cholesterol in ascitic fluid has been proposed to be a marker used to discriminate malignant ascites. Despite contradictory findings in previous studies, some reports have showed that

cholesterol concentrations in ascitic fluid are significantly higher in patients with peritoneal carcinomatosis compared to patients with ascites secondary to liver cirrhosis. In addition, this parameter was superior to other common markers such as total protein, LDH and SAAG to discriminate malignant ascites. Unfortunately, there is no standardized cut-offs and the previously reported values vary within a wide range (between 46 and 70 mg/dl) [35, 44].

Bilirubin

Normal bilirubin level in ascitic fluid is comparable to the serum level. The measurement of bilirubin in ascitic fluid can be useful when a bile leak secondary to intrahepatic or gallbladder fistula or upper gut perforation is suspected. A previous study based on a scarce number of patients showed a cut-off of 6 mg/dl of bilirubin in ascitic fluid as well as an ascitic/serum bilirubin ratio of >1.0 as being highly suspicious of bile ascites [45].

3. PARACENTESIS

3.1. General Principles

Paracentesis is an invasive procedure that consists of the extraction of ascitic fluid through percutaneous puncture of the abdominal wall for diagnostic and/or therapeutic purposes.

3.2. Diagnostic and Therapeutic Application

Technical features depend mainly on the purpose: to obtain ascitic fluid for analysis (diagnostic technique) or additionally to alleviate a massive ascites with large volume paracentesis (therapeutic approach). Diagnostic paracentesis is always indicated at the onset of ascites in order to diagnose the underlying disease as well as when ascitic fluid infection is suspected. Regarding the therapeutic approach, it is predominantly related to refractory

ascites due to liver cirrhosis. Nevertheless, management of refractory ascites is beyond the scope of this chapter, and the best clinical evidence-based recommendations have been recently addressed in several review articles [4, 12].

3.3. Preparation

Once informed consent has been obtained, the appropriate equipment should be assembled (Table 3).

Table 3. Equipment for abdominal paracentesis

Sterile gloves
10% povidone iodine solution or 2% chlorhexidine gluconate
Sterile gauze and drapes
Local anesthetic (1% mepivacaine)
Syringes: 10 and 20 ml
Needles: • 18-gauge needle for filling the anesthetic syringe • 25-gauge x 5/8'' needle for subcutaneous anesthetic • 21-gauge x 11/2'' needle for diagnostic paracentesis and for soft tissues anesthetic • 16-gauge x 2"safety IV catheter • 18- or 22-gauge, spinal needle for obese patients if required
Hematology (x1 tube); biochemistry (x1 tube); cytology (1-3 tube) if indicated
Two blood culture bottles (aerobic and anaerobic)
Reinforced Skin Closures ½ " x 4"
If therapeutic paracentesis: • Sterile closed drainage systems • Collector bottles or bags • If available, pre-packaged Commercial closed drainage suction system (it includes: 16-gauge retractable needle, sterile closed drainage system and drainage waste collector bag)

3.4. Procedure

Despite this being a routine procedure, the literature on the technical aspects of abdominal paracentesis is scarce, so the standard technique according to our local procedure is described as follows (Figure 1).

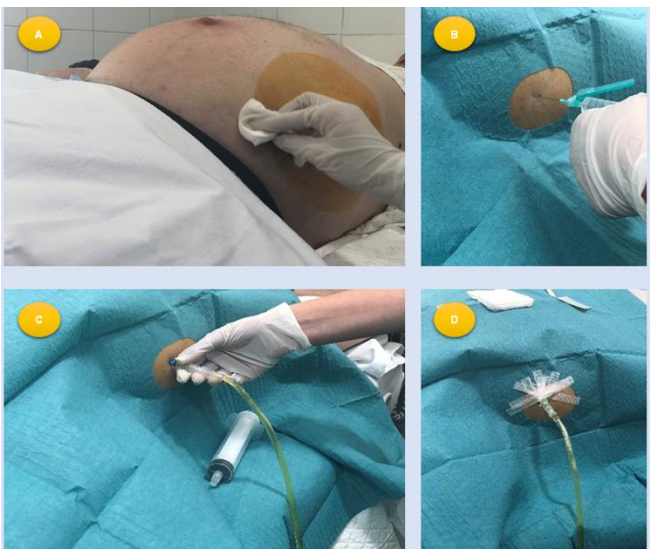

Figure 1. Therapeutic paracentesis procedure. A) Skin disinfection with antiseptic solution and sterile gauzes prior to covering it with sterile field. B) Local anesthesia administration (a total of 10 ml of 1% mepivacaine) first subcutaneously and then into the soft tissue (down to peritoneum). C) Puncture with a needle attached to a syringe or a closed drainage suction system. It is advanced directly perpendicular to the skin through the planned site up to peritoneal cavity. D) The system is attached afterwards with reinforced skin closures.

First, the patient should be placed in a comfortable position, which depends on the purpose of the procedure. Therefore, the supine position is preferred for LVP while lateral decubitus might be enough to obtain a single sample for diagnostic purpose.

Afterwards, identification of puncture site must be done through percussion on the abdominal wall to determine the level of dullness. If physical examination does not provide adequate information about the optimal site of puncture, an ultrasound is recommended for better localiza-

tion. Usually, in the left lower quadrant of the abdominal wall, at two finger widths (3 cm) cephalad and two finger widths medial to the anterior superior iliac spine, is the thinner point of the abdominal wall and with a larger pool of fluid so this is usually a good choice for needle insertion. The area of the inferior epigastric arteries should be avoided as well as visible collaterals [12].

After the puncture site is appropriately selected, the skin has to be cleaned with an antiseptic solution and sterile gauze prior to covering it with sterile field (Figure 1A). Local anesthesia (a total of 10 ml of 1% mepivacaine) is administered first subcutaneously and then into the soft tissue (down to the peritoneum). Aspiration with the syringe while progressing or moving back is necessary in order to avoid intravascular anesthesia administration (Figure 1B). Ascitic fluid aspiration indicates that the tip of the needle is inside the peritoneal cavity. While under the effects of anesthetic, an appropriate needle is attached to a syringe and is inserted and pressed forward perpendicularly to the skin through the planned site until the peritoneal cavity is reached (Figure 1C). The choice of the needle will depend on the procedure purpose and anatomic features of the patient. Then 30 ml of ascitic fluid, 10 ml for hematology and biochemistry analysis, and 20 ml for the microbiological one (10 ml for each blood culture bottle) are collected. Quick bedside inoculation of the ascitic fluid into blood culture bottles seems to enhance its sensitivity [19]. Once the desired amount of fluid is collected, the needle is removed while aspirating until it is completely out. Applying pressure to the puncture site to ensure no excessive bleeding or fluid leakage for a short period of time is advisable as well as changing the patient's position to the opposite lateral decubitus to the puncture site for a while (15-20 minutes) after applying reinforced skin clo-sures.

The clinician must ensure that all the samples are sent to the laboratory for analysis. When therapeutic paracentesis is performed, after the sample extraction as mentioned above has been done, a sterile closed drainage system has to be connected to the puncture device (Figure 1D). Once the desired amount of fluid is drained, the same procedure described above for needle removal in diagnostic paracentesis is done [1].

3.5. Risks and Complications

Paracentesis is a mandatory procedure when ascites is documented for the first time and when a complication is suspected. Several clinical conditions must be taken into account prior to the procedure, since there are relative and absolute contraindications due to an increased risk of complications.

The only absolute contraindication to performing a paracentesis is the presence of severe coagulation disorders such as clinically evident hyperfibrinolysis or disseminated intravascular coagulation. Despite this premise, there is no data-supported cut-off of coagulation parameters beyond which paracentesis should be avoided. Relative contraindications include pregnancy, severe bowel distension, abdominal skin infection at the proposed puncture site, and previous extensive abdominal/pelvic surgery. Nonetheless, the later can be managed by performing an ultrasound guided puncture [1, 4].

Although a high morbidity and even mortality rate related to the procedure has been described in the past, more recent publications regarding complications showed a low incidence of both major and minor adverse events, even in patients with coagulation abnormalities. LVP is a safe procedure to treat tense ascites, with less than 1% risk of complications [46]. The most common adverse events are usually mild such as abdominal pain in the puncture site or abdominal wall hematomas and do not require neither intensive treatment nor further complementary studies. Risk of mortality as a direct consequence of a major complication of paracentesis has been estimated in the literature as being less than a 0.5%, mainly in patients with extremely poor general conditions [47, 48]. Moderate and severe complications are discussed below.

3.5.1. Bleeding

Although hemorrhage is a potentially lethal complication, severe bleeding after abdominal paracentesis is very uncommon, and affects less than 1% of patients, even in those with coagulation disorders (i.e.,

International Normalized Ratio -INR- >1.5 or thrombocytopenia < 50.000 cell/µl).

A study of 410 ultrasound-guided diagnostic (23%) or therapeutic (77%) paracentesis performed between 2003 and 2005 and 163 patients in an emergency setting revealed only two cases of minor cutaneous bleeding. There were no significant procedure related bleeding events, even in patients with marked thrombocytopenia (14% of procedures were done with a platelet count less than 50.000 cells/µL) or coagulopathy (35% with an INR >1.5). The number of procedures carried out on a single patient ranges between 1 and 17. Interestingly, both patients who presented mild cutaneous hematoma showed platelet counts over 50.000cells/µL [49].

In a large case study of 4729 procedures along 10 years (1994-2003), the occurrence of severe hemorrhage represented 0.19% of all the procedures with a mortality rate of 0.016%. The mean MELD score of these patients was 30 ± 13 and the mean Child-Pugh score was 11 ± 2. Severe bleeding was defined as the presence of hemodynamic instability or a significant drop in the hemoglobin level (>1.5 gr/dL). Although there were only 9 cases of significant bleeding, it is important to note that 7 out of these 9 patients died within the first month after the index procedure (one of them died from hemorrhagic shock 10 hours after the paracentesis). Only 1 of these patients presented with a platelet count below 50.000 cells/µL, and 4/9 had an INR of < 1.5. Interestingly enough, 8 out of the 9 bleeding complications appeared in patients with some degree of renal failure; suggesting that perhaps the qualitative alterations of the platelets in this setting predisposes the patients to bleeding events [48].

This data has been further supported by a recent case-control study showing that renal dysfunction was the only independent predictor of bleeding after paracentesis (OR 4.3, 95% CI 1.3 - 13.5, p = 0.01), regardless of the MELD score, LVP, sepsis, platelet count, INR, and hemoglobin levels [50].

Another study including 1110 LVP performed on 628 patients (513 of whom were cirrhotic patients) between 1994 and 2001 did not find any major or minor bleeding complications, despite having platelet count as low as 19.000 cells/µL (with 54% of the patients under 50.000/µL), or an INR

as high as 8.7 (75% of the patients over 1.5 and 26.5% over 2). The procedures were performed in specific facilities in an endoscopy unit, without ultrasonographic guidance and without pre-procedural prophylactic transfusions of blood products. Up to 612 paracentesis were carried out safely with platelet counts below 50.000/μL. In one patient, with a platelet count of 64.000/μL, the ascitic fluid tap was initially hemorrhagic but subsequently clear [51].

A subsequent study from the same group, retrospectively reviewed, between 2005 and 2011, the electronic medical records of 205 patients with platelet counts less than 50.000 cells/μL who received ultrasound-guided paracentesis (304 procedures) performed in the Radiology Department, without pre-procedural platelet transfusions [52]. The objective was to evaluate evidence of major bleeding complications and their clinical sequelae. The mean platelet count was 38.400 ± 9.300cells/μL, and the mean INR was 1.6 ± 0.5. Only 3 major bleeding complications were observed (complication rate of 0.99%; 95% CI: 0.3%–2.9%) which occurred in patients with platelet counts of 41.000, 44.000, and 46.000cells/μL. All 3 patients were stabilized after blood transfusions without any additional interventions. Of note, all 3 complications happened in hospitalized patients with decompensated cirrhosis, and 2 of the patients also had dialysis-dependent renal failure. A subgroup analysis of bleeding rates included 267 patients with platelet counts of <50.000 cells/μL and INR > 1.5. Major bleeding complications occurred in 2 of the 149 patients (1.34%) with an INR of $\geq$1.5 and 1 of the 118 patients (0.85%) with an INR of < 1.5. The relative risk of major bleeding with an INR of 1.5 or greater among these patients was 1.45 (95% CI, 0.13–9.58). Thus, no statistically significant increased risk of bleeding associated to INR alteration was observed [52]. The low complication rate in this population, supports the theory that ultrasound-guided paracentesis can be performed without routinely checking or correcting serum platelet concentrations before the procedure.

In summary, the pooled data from the 4 most recent case studies published on this regard shows that severe bleeding complications occurred only in 12 out of 6553 paracentesis (0.0018%). However, special attention must be paid to patients with concomitant kidney dysfunction.

3.5.2. Pain

Pain is not a frequent event, neither during nor after the performance of LVP. When using a local anesthesia from the epidermis to the highly sensitive parietal peritoneum, with 1 or 2% mepivacaine, the procedure is practically, painless and clearly acceptable for the patient. Although uncommon, when abdominal wall hematoma occurs because of the puncture, the patient may suffer from non-severe and self-limited pain (24-48 hours), usually easy to control with first line analgesics such as paracetamol.

Importantly, patients must be advised to avoid using non-steroidal anti-inflammatory drugs as painkillers, especially patients with underlying decompensated cirrhosis. Although metamizole, also known as dipyrone has only a minimal anti-inflammatory effect, it does not have a favorable safety profile for decompensated cirrhotic patients, as it can aggravate their marked systemic hemodynamic derangement, as well as the related cytopenias [53]. In the rare case of more severe pain, the use of low power pure non-selective agonist of opioid receptors, such as tramadol, could be considered.

3.5.3. Infection

Paracentesis must be performed in a sterile manner after performing a surgical hand washing procedure and using sterile gloves. Although not essential, a surgical facemask and sterile gown may be considered. Cleaning the patient's skin with an antiseptic solution, and then applying a sterile drape is mandatory. Abiding by these recommendations, the risk of related puncture site infection is extremely low. Even when tunneled catheters are used for the treatment of refractory malignant ascites, with an overall median catheter life of 32 days, only 5% of patients developed mild local cellulites [54].

On the other hand, in a relatively recent series of 171 decompensated cirrhotic patients receiving 515 paracentesis over 24 months, there were only 3 episodes of bacterial infection, all of them secondary to therapeutic procedures (0.5%). During therapeutic paracentesis, the mean volume of ascitic fluid removed was 4390 ± 760 mL, and the largest volume of drainage was 20 L. Ultrasound was used in 11.7% of the cases to verify the presence

of ascites and the absence of adherent organs to the abdominal wall. The macroscopic appearance of ascitic fluid in one case was green-brown, strongly suggesting a procedure-related bowel perforation. Indeed, two patients presented with an iatrogenic small bowel perforation and one patient with an external contamination of ascitic fluid by staphylococcal bacteria. One of the patients that presented with a small bowel perforation died from secondary peritonitis [47].

Iatrogenic perforation of cecum induced by peritoneal catheter insertion has also been described and, interestingly enough, it could be successfully treated using the endoscopic clipping approach [55]. Although very unusual, bowel perforation is a life-threatening condition. In consequence, severe bowel distension should be carefully excluded before proceeding with paracentesis.

3.5.4. Persistent Leakage

Decompensated cirrhosis or malignant ascites is associated with poor wound healing. Abdominal wall injuries in these patients, even as minimal as a paracentesis puncture is challenging due to increased intra-abdominal pressure, risk of peritonitis, and ascitic fluid leakage.

There is very little data about persistent leakage following paracentesis. In an interesting series of cases, outflow of ascitic fluid through the puncture site was observed in 26 patients (5% of the cases). In four of them, the ascites outflow was significant enough to force stoma-pouch application until it spontaneously stopped. There were no reported cases of clinically relevant hypotension or renal dysfunction secondary to persistent leakage [47].

Persistent loss of fluid refractory to conservative treatment is uncommon, but when it occurs, a surgical closure of the abdominal wound using staples or stitches may be needed. Some case reports suggest alternative treatments to surgery such as topic adhesive glues [56]. The application of vacuum-assisted closure (VAC) therapy for postoperative ascitic fluid leaks through surgical wounds in cirrhotic patients have also been described [57]. VAC therapy improves angiogenesis and epithelialization, controls bacterial contamination, and removes excess tissue fluid. It could

be considered as an alternative for severe and intractable abdominal wall fistulae.

3.5.5. Post-Paracentesis Circulatory Dysfunction (PPCD)

Decompensated cirrhotic patients develop a progressive systemic hemodynamic derangement consistent in arterial hypotension and hyperdynamic circulation, mainly due to reduced effective volemia secondary to splanchnic arterial vasodilation. Consequently, sodium retaining systems, such as the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system become increasingly activated, leading to renal sodium retention, extracellular fluid volume expansion and ultimately ascites formation. Subclinical circumstances such as low-grade systemic inflammation, mainly derived from gut bacterial translocation, may severely aggravate and self-perpetuate this situation [58].

The removal of significant volumes of ascitic fluid during LVP may be associated to further circulatory dysfunction because of the sudden reduction of effective blood volume, leading to progressive arterial hypotension and organ hypoperfusion [59, 60] and increased plasma catecholamine and renin levels [61]. This condition known as post-paracentesis circulatory dysfunction may be progressive and self-perpetuating once established, with negative clinical effects [62, 63]. First, PPCD is associated with faster re-accumulation of ascitic fluid, requiring more frequent procedures and leading to subsequent circulatory dysfunction [64]. Additionally, the actions of vasoconstrictor systems on the intrahepatic vascular resistance increases portal hypertension [60]. Consequently, up to 20% of patients develop hepatorenal syndrome (functional acute kidney injury), dilutional hyponatremia and even hepatic encephalopathy [63], which is linked to low survival rates [62, 65].

Patients with severe hepatocellular insufficiency, as defined by hepatic or coagulation failures in the Chronic Liver Failure Consortium Organ Failure (CLIF-C OF) score are at a higher risk of developing PPCD-related Acute-on-Chronic liver failure (ACLF) [66]. As recently described, ACLF is a major driver of mortality in decompensated cirrhotic patients [67]. Strategies to avoid PPCD development will be discussed later in this chapter.

3.6. Prevention and Management of Complications

This section will mainly discuss and answer questions related to minimizing the risk and treating complications of paracentesis. So, we will address when it is necessary to guide paracentesis by ultrasound, when a transfusion of blood products is required, how we can prevent the PPCD and if there is any strategy to avoid persistent leakage.

3.6.1. When Is It Necessary to Guide Paracentesis by Ultrasound Imaging?

Experienced operators can safely perform paracentesis without echography guidance. However, ultrasound (US) is nowadays almost universally available to clinical physicians, so it is clearly advisable to routinely use echography, especially for residents that are learning. Indeed, US guidance has its advantages compared to the traditional technique. US is not only better for the detection of ascites when compared to physical exams [68] but also because it decreases the incidence of complications [69, 70]. Additionally, it seems to improve the rate of successful drainage, reduces the incidence of a dry tap and it can increase the probability of successful drainage after a failed blind attempt [71].

Accordingly, US guidance must be used in some situations:

- Diagnostic procedures where a small amount of ascitic fluid exists, or when there are doubts about the presence of ascites.
- Existence of multiple peritoneal septations or ascites loculation, which is frequently present in malignant ascites and up to 15% of patients with non-malignant ascites and previously repeated procedures [72].
- Puncture in the right lower quadrant, because of the proximity of the cecum.
- Small bowel loops distension because of ileum or intestinal obstruction.
- Dry tap, or history of prior difficult paracentesis.
- Previous complications related to this procedure.

- Malignant ascites to avoid peritoneal implants puncture and spreading.

A curvilinear US transducer with a central frequency range of 2.5-7.5 MHz is the apparatus most commonly used for imaging the abdomen [73].

The first step is to perform a general abdominal inspection to confirm the presence of ascites. In patients with tense ascites, this is unnecessary but it becomes mandatory in patients with mild ascites. Then, it helps to avoid injury of close viscera, such as kidney, spleen or cecum (in the lower right quadrant).

Once the ideal site has been selected, two measurements are necessary to determine the trajectory and depth of the puncture. First, the thickness of abdominal wall (especially in obese or oedematous patients) indicates the minimum depth required to reach the peritoneal cavity and it is useful as a safety check: if ascitic fluid is not obtained at the expected depth, the point of insertion and the puncture angle must be reconsidered. On the other hand, since the most likely structure at risk to be injured is the bowel, the vertical distance between the most superficial loop of the bowel and the inside border of the abdominal wall should be measured and never be exceeded with the needle. Interestingly, the expected amount of fluid to be drained (in liters) is approximately equal to this distance (in centimeters), called *smallest fluid depth* [74].

Although not mandatory, it would be advisable to confirm that there are no blood vessels in the selected route of insertion. Collateral portosystemic vessels are usually visible and easily avoided. However, to exclude the presence of arterial circulation a linear US transducer is necessary, usually in the range of 10-12 MHz [73]. The main vessel of concern is the inferior epigastric artery (IEA), which arises from the external iliac artery and runs cranially until its anastomosis with the superior epigastric artery. Typically, it is found approximately 5-6 centimeters from the midline on each side, but this distance can be quite variable, especially in obese patients. Scanning depth should be deep enough to allow visualization of the full thickness of the abdominal wall with a small amount of ascites visible at the bottom of the screen. The area around the planned procedure site should be scanned in

B-mode and with a color Doppler to exclude the presence of the IEA, which would typically appear as a pulsatile structure flanked by two inferior epigastric veins. Arterial flow can also be confirmed using a pulse wave Doppler.

Guided paracentesis with needle insertion under real-time US guidance is not needed in the majority of cases. Its main indications are the presence of a small amount of ascites or the puncture of intra-abdominal collections.

3.6.2. When Is the Transfusion of Blood Products Required?

Coagulation cascade in liver cirrhosis is pathologically rebalanced between circulating procoagulant and anticoagulant factors. Most procoagulant factors are found to be decreased in the plasma of patients with chronic liver failure. However, it is counterbalanced by a reduction in plasma levels of anticoagulants such as *antithrombin and protein C* [75].

In addition, some pro-hemostatic drivers such as *von Willebrand factor* [76] and *factor VIII* [77] are increased in patients with chronic liver diseases.

Because routine coagulation tests are frequently abnormal with prolonged coagulation times in these patients, it was traditionally thought that cirrhosis is *per se* a hypocoagulable state [78].

Nevertheless, the basic laboratory coagulation tests (i.e., prothrombin time, activated partial thromboplastin time, and thrombocyte count) can reflect a deficiency in the procoagulant factor but they cannot indicate whether this deficiency is accompanied by a concomitant deficiency of the anticoagulants. In fact, plasma coagulation in cirrhosis is not abnormal when assessed with global tests reflecting the function of both pro and anticoagulants, such as the thrombin generation test [75, 79]. Indeed, conventional tests poorly correlate with the risk of bleeding after a liver biopsy or other potentially hemorrhagic procedures [80, 81].

Blood product transfusions [fresh frozen plasma (FFP) or pooled platelets (pPTL)] are frequently used before paracentesis in patients with cirrhosis and abnormal coagulation tests. Conversely, a survey on the use of blood products before performing paracentesis showed that 50% of hepatologists never used FFP pre-procedure or used it only if the INR was severely impaired (>2.5) [82].

Prospective studies aimed to correlate conventional coagulation tests and the risks of bleeding following invasive procedures in cirrhotic patients are scarce. The risks and costs of prophylactic transfusions or thrombopoietin-receptor agonists might exceed the benefit.

Recently a prospective multicenter study evaluated the safety of different invasive procedures in the presence of abnormal coagulation tests, and the correlation between conventional coagulation parameters and significant bleeding in cirrhosis [81]. Three hundred and eighty patients were enrolled in the study and were divided into 2 groups, according to the presence or absence of abnormal coagulation parameters, which were defined as INR ≥ 1.5 or platelet count $\leq 50.000/\mu L$ respectively. None of the patients received pre-procedural FFP or pPTL. One hundred and twenty-eight patients (33.68%) were classified in group A (abnormal tests). Both groups were similar in regards to features other than coagulation tests and severity of liver disease. *Low risk procedures* (paracentesis being the most common) were carried out on 47% and 53% of the patients in groups A and B respectively. None of the patients, regardless the group, had clinically significant bleeding. *High risk procedures* (central vein cannulation, liver biopsy, polypectomy, Transjugular intrahepatic portosystemic shunt-TIPS-) were carried out on 14% and 10% of the patients, respectively. Three patients in group A (abnormal coagulation tests) developed clinically significant bleeding, a difference that is statistically non-significant.

On the other hand, a trial evaluating on the efficacy of eltrombopag for increasing platelet counts and reducing the need for pPTL transfusions in cirrhotic patients undergoing an elective invasive procedure, had to be prematurely stopped due to an increased risk of thrombotic events compared with the placebo [83]. Interestingly though, 50% of the patients were decompensated (Child-Pugh B or C). In a different setting, recombinant activated factor VII failed to improve the 24 hour control of bleeding, rebleeding or 5-day mortality in advanced cirrhotic patients (mostly Child-Pugh C) with active variceal bleeding, when compared to the placebo [84].

Thrombelastography (TEG) is a global hemostasis assessment device that measures the viscoelastic changes that occur during the hemostatic process, and provides a more comprehensive global coagulation assessment

than routine tests [85]. It is effective in guiding transfusions during liver transplantation [86, 87] and to monitor perioperative changes in coagulation during surgery [88]. Two TEG parameters robustly indicate the presence of coagulation derangement: first, the *reaction time* (the latency time between the beginning of the test and the clot formation as the tracing reaches an amplitude of 2 mm). The second variable is the *maximum amplitude* (the greatest vertical amplitude of the tracing) [85, 87]. A recent trial evaluated if TEG might avoid the unnecessary blood product transfusion in patients with cirrhosis and significant coagulopathy (defined in this study as INR >1.8 or platelet count <50.000/μL) [89]. Sixty patients were randomly allocated (1:1) to a TEG-guided transfusion strategy or standard of care (SOC). The TEG group received FFP if the reaction time (r) was >40 minutes or pPTL if the maximum amplitude (MA) was <30 millimeters. The endpoints were blood product use and bleeding complications. The baseline characteristics of the patients in the 2 groups were similar. Per protocol, all subjects in the SOC group received blood product transfusions, versus 5 in the TEG group (100% vs. 16.7%; $P < 0.0001$). Sixteen SOC patients (53.3%) received FFP, 10 (33.3%) pPTL, and 4 (13.3%) received both FFP and pPTL. In the TEG group, none of the patients received only FFP ($p < 0.0001$), 2 received pPTL (6.7%; $p = 0.009$), and 3 received both FFP and pPTL (not significant). Iatrogenic bleeding occurred in only 1 patient of the SOC group after LVP. So, the authors concluded that in patients with cirrhosis and abnormal coagulation tests, the TEG-guided transfusion strategy leads to a significantly lower use of blood products compared to SOC before invasive procedures, without an increase in bleeding complications. It is also remarkable that even in patients with significant coagulopathy post-procedure bleeding was uncommon, indicating that TEG thresholds could be re-evaluated. Unfortunately, although being a very useful and promising tool TEG is not routinely available in clinical practice.

The American Association for the Study of Liver Diseases and the European Association for the Study of the Liver recommends against the indiscriminate use of FFP or pPTL therapy before paracentesis, as this policy is not data-supported [4, 12]. Both guidelines preclude paracentesis only

when there is clinically evident hyperfibrinolisis (major ecchymosis or hematoma) or disseminated intravascular coagulation [79, 81].

In conclusion, bleeding after paracentesis is a very infrequent event, although it can be life-threatening. A routine transfusion policy is not currently evidence-based, and it is not without inherent risks. A new tool such as TEG is probably the most promising future perspective to evaluate bleeding risk in cirrhosis. Until there is clear evidence regarding this issue, a careful individualized evaluation has to be adopted.

Another controversial point is the optimal strategy with patients under anticoagulant treatment. Often, the underlying disease responsible for ascites development predisposes the patient to an increased risk of thrombotic events, particularly in malignant diseases, cardiologic disorders, intraabdominal complications and even in cirrhotic patients, whose risk of thrombotic complications such as venous thromboembolism and splanchnic vein thrombosis is not negligible [90]. Therefore, many patients eligible for diagnostic or repeated large volume paracentesis might be on anticoagulation therapy [91].

There are no evidence-based recommendations/guidelines on the safety and the level of anticoagulation under which paracentesis can be performed. Some reports suggest that paracentesis is a low risk procedure even in patients with an active anticoagulant treatment. A recent single center study showed that paracentesis was a safe procedure in a group of 30 patients with a previous diagnosis of Budd-Chiari syndrome and on anticoagulation therapy. A total of 51 procedures were performed, on 26 patients for therapeutic purposes, and neither prophylactic blood products transfusions were administered nor were any US guided punctures used. The mean INR at the time of paracentesis while on anticoagulation was 3.1 (Range 1.4–7.9). No clinically significant bleeding events were reported (overt sign of hemorrhage over the puncture site, including abdominal wall hematoma or hemorrhagic into ascitic fluid with a drop in hemoglobin of >1.5 g/dl following the procedure). Of note, the mean platelet count was 220 ± 190 (10^3/ml) and all the patients had serum creatinine levels within the normal range [92]. Nevertheless, multicenter controlled studies are needed to support these findings.

The clinical decision regarding the safety of anticoagulant treatment discontinuation, and the need for bridge therapy or invasive procedure preclusion must be based on an individual rationale taking into account several factors: Indication of antithrombotic therapy, inherent bleeding risk of the procedure, periprocedural thromboembolism risk, type of antithrombotic agent (anticoagulant/antiaggregant), patient history and associated comorbidities such as renal dysfunction. The heterogeneity of the target population makes it difficult to provide a general recommendation in this regard. Some expert consensus reports have been published in order to facilitate clinical decisions on periprocedural antithrombotic therapy management (not specifically paracentesis), based on the individual risk/benefit profile of each patient [91, 93-96].

3.6.3. How to Prevent the PPCD?

As previously mentioned, paracentesis substantially influences systemic hemodynamics. Indeed, LVP may precipitate PPCD, which is associated with acute kidney injury and an increased risk of mortality.

It was first demonstrated in the 1980s that adjunctive albumin infusion favorably influences circulatory function after LVP and prevents the subsequent reactivation of vasoconstrictor systems and the occurrence of PPCD [63]. Both European and American clinical practice guidelines recommend the administration of albumin when the volume of ascites removed during paracentesis exceeds 5 L [4, 12]. Since the early 1990s, less-costly alternatives to albumin have been sought, such as vasoconstrictors and artificial colloid volume expanders, like dextran-70 (8 g/L of ascites removed), polygeline (150 ml/L) or saline solution (170 ml/ L). However, they only show a similar efficacy to 20% albumin (8 g/L) when less than 5 L of ascites are removed. Despite numerous randomized trials, it remained uncertain whether the effectiveness of such alternative treatments is comparable to that of albumin. Nevertheless, a meta-analysis of 17 trials involving 1225 patients demonstrates that albumin is superior to any other plasma expander or vasoconstrictor in this indication [97]. Effect sizes were substantial, with albumin reducing the odds of PPCD by 66%, hyponatremia by 42%, and death by 36%. Moreover, albumin administration was asso-

ciated with 15-19% reductions in the odds of ascites recurrence, renal impairment, and hospital readmission. Studies have infused between 5 and 10 grams of albumin per L of ascites removed; 6-8 g/L have been the most common used doses. One study did compare albumin doses in 70 patients; the 4g/L group had similar PPDC and renal impairment to the 8g/L group. However, 6-8 grams per liter of ascitic fluid removed is the most common dose administered and recommended. It would usually be infused during and/or shortly after the paracentesis [4, 12].

The superiority of albumin over the other alternatives may be related to the oncotic and non-oncotic properties of the molecules, providing pleiotropic effects associated to clinical benefits [98].

3.6.4. Is There Any Strategy to Avoid Persistent Leakage?

The most common maneuvers to reduce persistent leakage are applying pressure to the puncture site for a short period after needle removal, or a simple postural procedure of ensuring that the patient rests on their right lateral decubitus (the opposite side of the puncture side) for thirty minutes after concluding the procedure.

It has been suggested to employ the "z-track technique" in order to avoid persistent leakage after paracentesis [11]. The Z-track technique is a method of administering intramuscular injections in which the epidermis layer of the skin and the hypodermis layer of the subcutaneous tissue are moved 2 to 3 cm to the side or downward on the side of the non-dominant hand. This technique forces the entry path of the needle into a zigzag shape, thus preventing the drug from coming back out of the muscle tissue [99].

Its beneficial effects for the patient, mainly reducing pain and drug leakage, have been studied predominantly in the nursery field, but there is no available information in the literature of this technique regarding paracentesis performance.

4. FUTURE PERSPECTIVES

Since paracentesis is an invasive diagnostic and therapeutic method associated with adverse events, alternative options may be attractive.

4.1. Diagnostic Approach: Can Diagnostic Paracentesis Be Avoided?

Despite the huge evolution of many diagnostic tests over the last decades in modern medicine, and the highly sophisticated technology available, no diagnostic tool can replace the diagnostic paracentesis yet. Therefore, although the performance of other diagnostic assays (blood test, imaging techniques...) is still essential to fully characterize the underlying disease responsible of ascites, this procedure is still the most valuable tool and a cornerstone of the diagnostic work-up.

4.2. Therapeutic Approach: Can Large Volume Paracentesis Be Avoided?

Conversely, and specifically for the management of refractory ascites, several devices have been developed, tested and improved recently, mostly in the liver cirrhosis scenario, aiming to replace LVP. On one hand, devices based on a pathophysiological approach such as TIPS, and on the other hand, devices with a palliative intention such as automated low flow pump systems (Alfapump® system), or indwelling permanent peritoneal catheters are potential alternatives as we will see later.

4.2.1. TIPS

First developed in the late sixties in animal models and then later applied for the first time in 1982 in cirrhotic patients, TIPS has become, after more than thirty years, an essential tool for the management of refractory ascites, as well as other complications related to PH.

A transjugular intrahepatic portosystemic shunt consists of the creation of an artificial connection between portal and suprahepatic veins by inserting a stent aimed at decompressing the portal system and therefore, decreasing the portal pressure (Figure 2) [100]. Several randomized controlled trials, as well as meta-analyses with pooled information of the previous studies have been published during the last two decades. The first studies compared unco-

vered TIPS with the standard therapy for refractory ascites, that is, repeated LVP, the results showed that the former was better than LVP in preventing ascites recurrence but with a higher incidence of hepatic encephalopathy post-TIPS. Additionally, there were no differences in survival rates between both techniques [5, 101-105]. Nevertheless, after the first publications, a new meta-analyses using individual patient data of the same studies, showed a significant improvement of transplant-free survival in those patients treated with TIPS, which was strongly influenced by three prognostic variables (age, bilirubin and sodium) revealing the importance of careful patient selection [106].

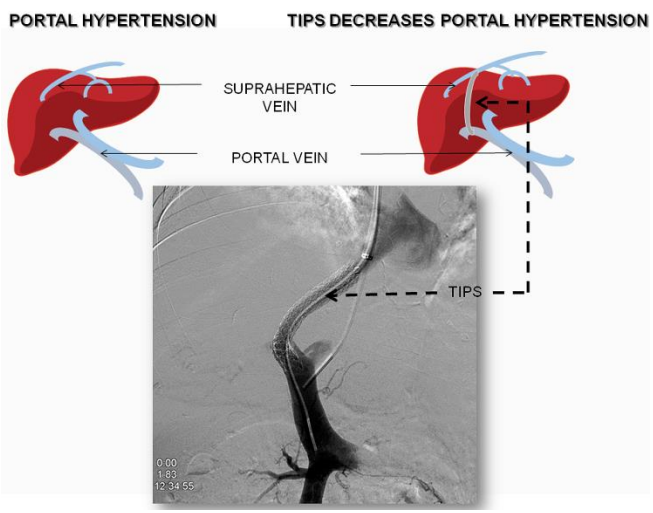

Figure 2. Schematic representation of TIPS: connection between the suprahepatic and portal venous system through an intrahepatic stent.

The release of the covered stents, which actually replaced the uncovered devices, suggested a great improvement in terms of less prosthesis dysfunction, decreased ascites recurrence, hepatic encephalopathy and better survival rates, this latter in turn, was also influenced by prognostic variables such as MELD [107].

Recently, a controlled randomized clinical trial compared, for the first time, both strategies accepted nowadays for refractory ascites treatment in

cirrhosis, repeated large volume paracentesis vs. TIPS with covered stents. The results of this study showed a better one-year transplant-free survival rate in TIPS group (93% vs. 52%; p = 0.003) as well as a lower incidence of treatment failure and, without a significant increase in the risk of hepatic encephalopathy [108]. Nevertheless, this study included a highly selected group of patients with relatively preserved liver function in whom ascites fitted better with the definition of recidivant instead of refractory [109]. This selected population limits the generalization of the results to the whole spectrum of cirrhotic patients with medically untreatable ascites.

According to the available scientific evidence, in selected cirrhotic patients, TIPS has demonstrated to be a safe treatment with better control of ascites, increasing the transplant-free survival rate, and consequently, replacing the standard therapy with repeated LVP.

4.2.2. ALFAPUMP® SYSTEM

A new device for patients with refractory ascites, not eligible for TIPS, was developed in 2011 to remove ascites from the peritoneal cavity into the urinary bladder. Ascitic fluid is then eliminated through normal urination, and it aimed to decrease paracentesis requirements. The Alfapump® system is a subcutaneously implanted battery-powered device with internal sensors that monitor the pressure in the peritoneal cavity and bladder in order to prevent pump operation when the peritoneal cavity is dry and bladder becomes full (Figure 3). It activates every 10–15 min, excluding the night time period, and moves 10–30 ml of ascitic fluid into the bladder depending on the programmed amount. It is implanted by minimally invasive surgery and requires to be charged for about 20-30 minutes every day.

The first multi-center study carried out on forty patients to evaluate its safety and preliminary efficacy showed that this system was associated with a significant reduction in the number of LVP from 3.4 per month (range 1–6) to 0.24 (range 0–5), while 40% of the patients had no paracentesis after receiving their pump. Patients had no significant liver, renal or hemodynamics derangement in this study. However, the device was found to be associated to many adverse events both technically and infectious,

which decreased along the study as technical and medical improvements were introduced, but still not negligible [110].

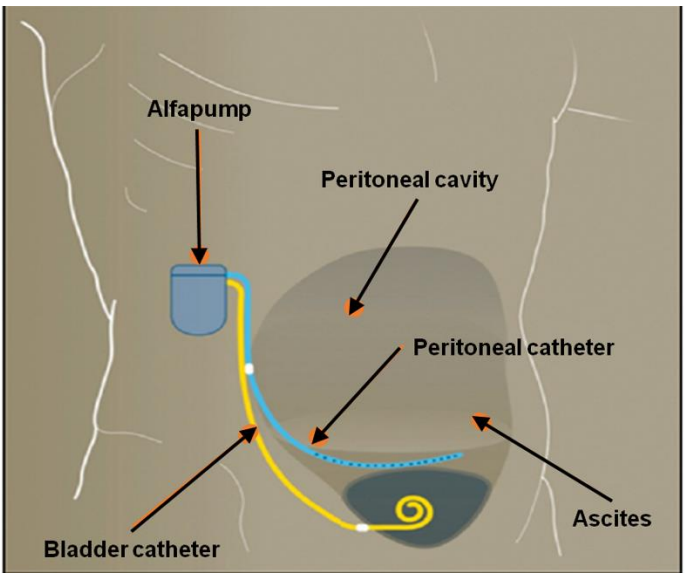

Figure 3. Schematic representation of an Alfapump system (Adapted from Bellot P. et al. with permission [110]).

Data from the first randomized controlled study, which included sixty patients allocated to either Alfapump® system or LVP, has been recently published [111]. The study showed a significant reduction in LVP requirements in the device arm as well as a significant improvement in quality of life assessed by Chronic Liver Disease Questionnaire (CLDQ) scores compared to LVP patients. Improvements in nutritional parameters such as handgrip strength and body mass index were also observed in a sub-study. No differences in survival rate were found between both groups. However, adverse events were significantly more frequent in the Alfapump® system group, many related to device mechanical complications that required surgical intervention in almost 45% of the cases, and infectious complications. Renal and urinary disorders, mostly acute kidney injury, were also reported within the first days of the implantation [111].

Although promising, it seems to be a therapy that needs to be technically optimized in order to reduce the adverse events.

4.2.3. Peritoneal Catheters

Several peritoneal catheters are available nowadays to mobilize refractory ascites, such as tunneled catheters, peritoneal ports and peritoneovenous shunts. A brief description of each technique is provided below (Figure 4).

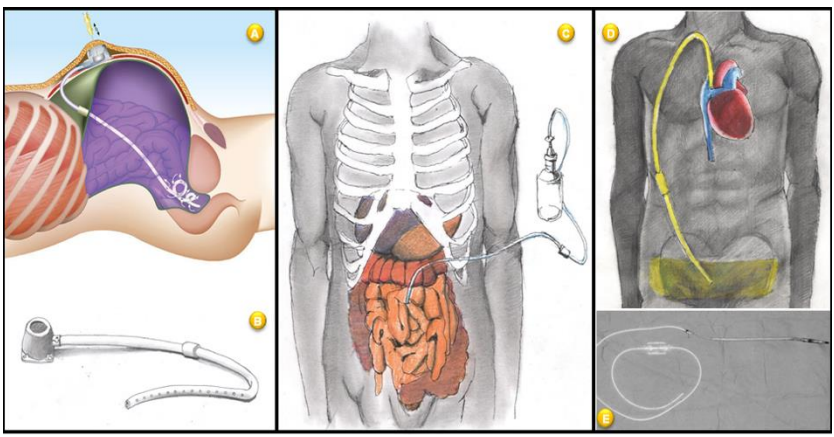

Figure 4. Peritoneal catheters. A and B: Peritoneal ports. C: Peritoneal tunneled catheters. D and E (Adapted with permission from Hussain FF et al. [114]): Peritoneovenous shunts.

- *Peritoneal ports (Figure 4A-4B):*
  Port-a-Cath peritoneal implantable access system is a portal and a catheter, similar to central venous access ports, but larger. The portal is placed under the skin to be accessed with a Huber needle. The catheter is fenestrated along the intraperitoneal portion and attaches to the side of the port. The catheter also has a cuff for the portion in the tunnel to ensure the catheter and to protect against tunnel infections. Once the catheter is in place, the technique for port access is similar to that for central venous chest ports. In sterile conditions, the port is accessed with a Huber needle (19 gauge; Smiths Medical) to achieve higher flow and to shorten aspiration

time, which is connected to vacuum bottles. The maximum aspiration volume recommended is 3 liters to avoid volume depletion. The port is flushed with 20 mL of heparinized saline solution (2,000 IU heparin) after each use [112].

- *Peritoneal tunneled catheters (Figure 4C):*
  Peritoneal tunneled drainage catheters consist of a fenestrated silicone catheter with a one-way valve mechanism and a polyester cuff. Once the catheter is in place, ascites can be drained when necessary by attaching a vacuum bottle to the catheter and draining fluid from the peritoneal cavity. Once the drainage is finalized, the bottle is disconnected and the catheter is kept underneath a dressing [113].
- *Peritoneovenous shunts (Figure 4D-4E [114]):*
  Briefly, peritoneovenous shunts consist of a connection, between peritoneal cavity and central venous system (jugular or subclavian) through a catheter that is surgically or radiologically put into place. The catheter has a single or double valve. One-way (unidirectional) valves prevent the reflux of blood into the venous limb of the shunt. Regarding the double valve iteration, the first valve opens to flow when the pressure exceeds 3 cm-water (cm H_2O) while the second valve acts as a "check valve" to prevent reflux of ascitic fluid or blood from the venous limb into the valve chamber while it is "pumping." Therefore, the two-valve model is more effective at preventing reflux and is the shunt most commonly used. The one-valve shunt is especially useful when ascitic or pleural fluid is very viscous or when the daily production of ascites is large [115].

All three devices need the appropriate training of both patient and caregivers, in addition to periodic follow-up intervals with close nursery support.

A recently published review compiling the available evidence from 29 studies, heterogeneous in their methodology, evaluated these three different drainage systems. Patients included had refractory ascites due to both benign and malignant underlying diseases. From a technical perspective, a technical

success of 100% was reported for tunneled catheters and peritoneal ports. Lower success rates are associated to peritoneovenous shunts insertion. Regarding patency, defined as the time of a working catheter in situ, tunneled catheters and peritoneal ports studies reported of patency of >250 days for single individuals while less information was available from peritoneovenous shunts studies with a reported patency in one study of 65% at the time of death. About the complications, not all the studies provided detailed information and definitions were heterogeneous. Nevertheless, the most frequent adverse events reported were peritonitis (especially in tunneled catheters and peritoneovenous shunts), cellulites (more frequent in peritoneal ports), and ascites leak (overall for tunneled catheters and peritoneal ports). Severe complications are uncommon and usually noticed in the studies related to peritoneovenous shunts, among others, pulmonary edema and pulmonary embolism, gastrointestinal bleeding, disseminated intravascular coagulation, encephalopathy and heart failure [116].

Therefore, peritoneal catheters as a palliative treatment are a feasible and relatively safe option in patients with malignant related ascites, and also, patients with benign ascites who are not candidates for other definitive therapies.

CONCLUSION

Ascites is always a relevant finding and elucidating its origin is mandatory, although in up to 80% of cases, it is related to liver cirrhosis. Paracentesis is a cornerstone in the diagnostic work-up of ascites. It is an easy procedure associated with a low incidence of complications, even in patients with coagulation disorders. Despite this, complications may occur, although severe events are seldom observed, so strategies aiming to minimize these risks have been reported, including, US guided puncture, albumin infusion after LVP to avoid PPCD and blood products prophylactic transfusions in selected cases. Besides its relevance to clarify the underlying disease, paracentesis also plays a critical role in patients with tense ascites in order to relieve secondary dyspnoea, anorexia, and abdominal discomfort.

Although no diagnostic tool is able to replace the diagnostic paracentesis that has been developed so far, different strategies and devices are available as a potential alternative to LVP for patients with refractory ascites.

CONFLICT OF INTEREST

There are no conflicts of interest.

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Chapter 2

TENDINOPATHY: AN UPDATE FROM THE PERSPECTIVE OF THE PHYSIOTHERAPIST

Ana Gacimartín-García, MD[1], Luis Prato, MD[2] and Luis Espejo-Antúnez, PhD[3,*]

[1]FREMAP Working Accidents Insurance Company, Alcobendas, Madrid, Spain
[2]Lakeshore Bone & Joint Institute, Portage, IN, US
[3]Surgical-Medical Therapy Department, Extremadura University, Badajoz, Spain

ABSTRACT

To this day, the knowledge provided by scientific research regarding tendon injury is minuscule in comparison to what is left to understand about it. However, there is much more known now than there was just a short while ago. In the past two decades, researchers have given an important turn regarding these injuries.

This is why it is a shame that when most clinicians say "tendon," the only thing that they still relate it to, is with treatment through eccentric exercise in best case scenarios, if not with passive models of manual

* Corresponding Author Email: luisea@unex.es

therapy. Advances in science should suppose changes in our clinical practice, and this new information is starting to be heard in the community of physical therapists closer to current research, but it is not an extended knowledge, and even less, used regularly in our daily practice with patients that suffer this type of problems.

This is why in this chapter we will summarize what we should be doing today about injuries of the tendon and how to approach them in a concrete and schematic matter.

Keywords: tendinopathy, physical therapy, physical exercise

1. TENDINOPATHY CONCEPT AND PATHOPHYSOLOGICAL MODELS

To define the concept of tendinopathy there are three types of models according to their etiopathogenesis [1]:

1.1. Inflammation

The term tendinitis was originated in the 70s, when the injury was considered the result of a chronic inflammation [2]. This assumption was summarize lately in the inflammatory model [3], which suggested that a process of inflammation in small acute episodes, with repetitive micro-trauma that form small areas of scar in the tissue and, ultimately, its rupture. The classic inflammatory response had been observed in tendons which suffer ruptures or lacerations [4], while in pathological tendons he has been observed inflammatory cells [5] but the response does not look like the traditional inflammation.

The presence of these cells do not necessarily mean that the inflammation is the primary event or the driving mechanism for the tendon pathology [1].

1.2. Collagen Degeneration

The dogma of inflammation was later largely questioned [6, 7, 8], and in the 90s *the failed cicatrization model* was proposed [9], which describes the tendinopathy as a process of failed cicatrization. There was an increase of cells and protein synthesis in the tissue, but with disorganization in the matrix along with a process of neovascularization. Meaning that the tendon tried to heal but couldn't do it, provoking a chronic state of tissue degeneration. This is why from there we went to the term *"tendinosis,"* emphasizing this process. But this model was debated as well. The collagen fibers in vivo cannot be broken without substantial alterations in the non-collagenous matrix and their studies in animal models have limited relevance in human tendinopathies [10].

1.3. Cellular Response

The difficulty of animal tissue studies made us accept that we did not know exactly what was happening in the tendon injury and therefore "Tendinopathy" [11] was proposed as the correct term to use, since it refers to tissue dysfunction regardless of the pathology that is being produced. It was then when in 2009 when Jill Cook & Purdam gave a new light on this debate through their research, publishing in this year the Continuum Model [12] which explains the tendinopathy in three phases:

1.3.1. Reactive Tendinopathy

When the healthy tendon receives an overload, it is forced to provoke a drastic reaction to try to "get out of the way" until it has time to adapt: a non-inflammatory proliferation of the cells and the tendon matrix that increases its transverse thickness. Why? To reduce the load of both tensile and compressive forces increasing its surface. That is, the tendon will moderate the load by itself in an urgent manner until it has time to adapt. This is short term adaptive response of the tendon and is strongly demonstrated in vitro models [12], being a different process to that which occurs in the normal

adaptation of the tendon when the loads are physiological, which is produced by increasing the rigidity of the tendon fibers with minimal changes in thickness.

Clinically, reactive tendinopathy is due to acute overloads, usually because of physical activity that is not familiar or after resuming it after a break. It could also be due to direct hits. It is common in young patients and, with the appropriate treatment, is completely reversible.

Imaging tests usually show a tendon with a fusiform shape and an increase in diameter, showing hypoechoic areas between intact areas of collagen in ultrasonography (US) and minimal or no increases in nuclear magnetic resonance (NMR) signals.

1.3.2. Tendon Disrepair

This phase is the tendon's attempt to healing, similar to the reactive one, but with the difference that there is already a greater degradation of the cellular matrix. There is an increase in the number of cells resulting in a significant increase in the production of proteins. In addition, there is an increase in vascularization and innervation.

Clinically this phase is difficult to distinguish from the reactive one. It is common in patients of a wider age spectrum, with young people and athletes being the most frequent, with a history of chronic overload (what happens when, for example, a patient has been suffering from tendon pain for months but takes medication and continues with his usual sports practice). To observation, tendons are already thickening and their possibility of healing is still possible, although in a smaller percentage.

Imaging tests will already show in this phase an evident thickening of the tendon as well as a disorganization of the collagen and the matrix. In US small areas of hypoechogenicity, and in Doppler, neovascularization becomes evident. In NMR the thickening of the tendon is seen, this time with an increase in the signal.

1.3.3. Degenerative Tendinopathy

In this phase the tendon is, literally, the remains of a battlefield. Cells have given up in their war for trying to heal and have retreated due to

exhaustion of tenocytes and apoptosis, leaving areas of cell death. The matrix has been disorganized and filled with new vessels but little collagen is left.

Clinically this phase is observed more in older patients, but it can also be seen in young people with a chronic overload of the tendon taken to an extreme in time. The ability to reverse structural changes is already small when there is cell death, but if something is not done in that small margin, the tendon can inevitably reach rupture.

Imaging tests will show hypoechoic regions with very few fascicles of collagen (US) and increased signal and tendon size (NMR).

At first glance we could think that this model does not differ from that of the term "Tendinosis" because it is also a failed healing. The difference is that Cook does not talk about terms that suggest a non-repairable pathology. It does not describe a model that goes directly from a healthy tendon to the degenerative phase, but speaks of an evolution in time in which adding or removing load will be the stimulus that will cause us to go forward or backward in this continuum, especially in the first phases.

If we contrast them correctly, the inflammatory model is not exclusive with the continuum. The tendinous cells are mechanosensitive, so inflamematory reactions in response to overloads stimulate the matrix by remodeling it (degradation and synthesis).

To facilitate the use of this model in the clinic, a classification divided into two types was created: reactive tendinopathy and degenerative tendinopathy, allowing a quick clinical classification of the cases in one of the groups based on the evaluation. An older person with a thickening of the tendon is likely to have a degenerative tendinopathy. A young athlete with a recent overload in her history is likely to have a reactive one. There will be cases in which the clinical presentation is difficult to frame, in such cases imaging tests will be key.

It is also important to consider that, while the continuum model is based primarily on the structure, it does not mean that there is a direct relationship between structure, function and pain. There are frank associations and dissociations between these three variables. Tendon pain is partially related to function, while changes in function can be related to changes in the

structure independent of whether pain exists or not. The three variables can be independent or coexist in binomials or a trinomial, without even understanding their exact relationship [1].

In any case, the debate is still open and more alive than ever. There are authors like Fu, who contemporaneously with Cook & Purdam have defended the model of failed healing [13]. And others, like Abate, who even support the inflammatory model [3]. This author argues that both inflamemation and degenerative changes coexist in the course of tendinopathy, without excluding one another, citing that the three classic phases of the healing model that have been described for other tissues (inflammatory, proliferative and maturational) are the same as in the tendon:

1.3.4. What Is the Current Accepted Model Then?

The Cook & Purdam model is currently the one with the highest degree of evidence, but it is not an absolute truth. There is still more research to be done to integrate these models and to know if both coexist in some way. But meanwhile, the Cook & Purdam model is the one that has the most scientific value, and therefore the most indicated for clinical use.

2. ETHIOPATHOGENESIS: MECHANOTRANSDUCTION CONCEPT

To understand the etiopathogenesis of tendon injuries, it is imperative that we first understand that of overuse injuries in general. As with any injury, it is critical that physiotherapists always think first of the causes before thinking of solutions. But in the tendon, as we will see, by its characteristics, it will be even more so. For this we will devote this section to underpin the concept of mechanotransduction.

Mechanotransduction is the process of cellular transformation in response to mechanical stimuli. That is, how our living tissues adapt and change their structure according to the loads they are subjected to. As physiotherapists we have tools such as electricity, sound, temperature... and

one that is as powerful as it is belittled in the inculcated models of manual therapy and called load. The handling of the load gives us an extremely useful technique: mechanotherapy.

Let's look at an example with muscle mechanotransduction, which is where we usually have this concept. The physiological threshold of a muscle, that is, the capacity it has, is between 4 and 5 kilograms. If I do not change my normal activity and I keep lifting objects of that weight, that threshold is maintained. This is what we call homeostasis.

But suddenly I fall ill and spend a few weeks in bed. Since I am not using this capacity of the muscle, my body, which is a "business" with a highly economic mentality since its main objective before any other is survival, decides that it is not profitable to maintain these muscle fibers since I am not using them. The physiological threshold of my muscle falls in response to this lack of stimulation and now only has the capacity to lift 2 to 3 kg: this is what we call atrophy.

I decide then, when I recover, to start going to the gym and start lifting weights slightly above the physiological threshold with which I was left. My body detects the need to put more fibers in that muscle because now I am using it and I am missing it. The physiological threshold rises and my strength increases in response. Now I can lift around 7 kg. The muscle has adapted to the stimulus and, therefore, has been strengthened.

But, what happens if in this progression instead of starting to challenge my threshold with dumbbells near my physiological threshold I decide to do it by taking the 20 kg ones? This is what we call, not load, but overload. And because the step is so high, my muscle fibers do not have the ability to adapt because, with such a high load, the war between destruction / healing is not feasible. Due to the lack of progression, my fibers break and I injure myself. And what happens to the threshold? It will lower because now I've destroyed the fibers

MECANOTHERAPY:
FORCE → CELLULAR DEFORMATION → ADAPTATION
According to dose and physiological threshold:
Improvement/Maintenance/Atrophy/Injury

Basically, we are always talking about a loading phase and a tissue adaptation phase. And that the load can be anabolic or catabolic according to the dose. This is applicable to all tissues.

When we apply mechanotherapy in any overuse injury we have to know what the graphic of that tissue has been, and our approach will always be similar to this:

- *1st: Moderation of the load.* If the tissue is subjected to a load above the threshold, that is, if it continues to be overloaded, there will be no possible recovery. But be careful, because if on the other hand the load is much lower than the threshold, it will continue to fall as well. What it is indicated then, will be to moderate the load so that it is neither superior nor inferior to the current threshold of the tissue so that, in that destruction-healing battle, it can begin to win and not continue to fall. That is, so that it can begin to heal.
- *2nd: Once the tissue is healed,* it is when loading should be increased to restore the previous threshold. This phase is often forgotten in the clinic, when the symptoms disappear, and the patient is often told to return to normal activity, which could potentially lead to a relapse.

Utilizing the mechanotransduction model to the tendon, and mechanotherapy as its therapeutic version, this model is critical for this type of tissue due to a primordial reason: because the tendon is very slow adapting. The tendon is a tissue with a slower metabolism than others. Slower than the muscle, the fascia, the ligament... and therefore it takes longer to repair and adapt than other structures. It was always said that this was because it was a tissue very poor in vascularization, but today it is known that in reality that is not true. We know now that the tendon is poor in cells, and the vascularization is not more than the one proportional to this number [14]. And this is the reason, and not another, why its metabolism is slow and, therefore, the speed of adaptation to the load is slower.

This is why, it is usually the tissue that, when the increase in the exercise load is not progressive, is susceptible to be the first one to be injured. And

in a society where the sports, recreational, work or domestic levels are not characterized by the word "slow," the tendon is an area that is commonly affected.

The most important thing at the clinical level is to take into account that, as in the overuse injuries of other tissues, tendon problems are mechano-transduction problems, but in the tendon we will have to take into account their slow speed, both in the treatment as above all and, very critically, in the return to activity.

3. Clinical Manifestations and Diagnosis

"Tendinitis" in the course of decades and unfortunately still to this day, or "Tendinopathy" in cases of greater language correction, has had a poor definition: any pain that cannot be diagnosed by an X-ray. And of this we have abused and continue to do so both doctors and physiotherapists. Therefore, the first thing we need is a differential diagnosis [15]. When we have a true tendinopathy in the clinic, these three points will always be met:

1) Pain localized with the tip of the finger: tendon pain is very localized, usually marked on the middle portion of the tendon or on its insertion to the bone. If the patient refers to a larger region it may not be a tendon problem, or it may be but accompanied by something else [16]. It is also a pain that is felt with the movements and very rarely at rest.
2) Intermittent pain (On/Off): tendon pain is especially pronounced when we stand for a while and move again. The maximum expression of this situation is in the morning when we wake up after several hours of sleep, being therefore the morning stiffness a very characteristic criterion of tendon problems. The first movements are usually very painful and as it warms the pain decreases (the so-called "warm-up phenomena") [17].
3) Change in the loads: in the history of the patient, we will always find a step up in the progression of activity that he has carried out. It may

have increased in an exaggerated way, or have often stopped and resume it (beginning of the season after the summer). This is characteristical of tendon problems because of what we have explained about their physiology. And we will always talk about two types of loads: the tensile, longitudinal to the tendon, to store energy and return it (characteristic especially of tendons of the lower limbs), and the compressive, perpendicular to it, that are produced in the physiological movements by the pressure against certain bony prominences (compression of the Achilles against the fulcrum of the calcaneus in the dorsiflexion, or supraspinatus against the acromion) or by external agents (very tight shoes). The latter usually cause insertional injuries.

If all three criteria are met, we truly have a tendon problem. If any of these three is not met, we probably have to continue digging deeper and differentiate with other structures.

And, is it not necessary to do some imaging test? The diagnosis of tendinopathies is clinical. This means that, as in other musculoskeletal syndromes, imaging tests are not necessary in a first evaluation except for those cases in which, due to the findings, we should rule out other problems such as fractures, complete or partial tendon ruptures, arthritis, calcifications, etc. [15]. Imaging will also be necessary in the reviews of those cases in which the evolution is not being satisfactory.

The diagnosis of a tendinopathy can never be made by the isolated result of an imaging test. For example, if the anterior area of the knee hurts and changes in the structure of the tendon are visualized when passing the ultrasound, this finding alone is not valid in any case to diagnose a tendinopathy. Why? Because if we did that test to healthy people, 50% would visualize a structural alteration [18]. Also because we could find people with tendons that meet the three previous conditions and their image is normal [19, 20]. Namely, as we have said previously, we cannot establish a direct relationship between structure and symptoms (in addition to function) [1].

In fact, it is curious to read studies where subjects with tendinopathy have experienced a decrease in pain of 80% at 12 weeks of treatment, while

there has not been a single change in the image until week 24 [15] supporting the idea of that there is no direct clinical relationship between pain and structure [21, 22]. This is explained because, to this day, we still do not know the mechanism of tendon pain [23]. That is why guiding us only through imaging does not help us to make a diagnosis.

But does this mean then that the image has no value? Absolutely not. That we cannot establish a direct relationship between structure and symptoms does not mean that it does not matter. In fact, and starting from the principle that "structure governs function," changes in the image are a predictor of risk to develop a tendinopathy or reach a complete rupture. As we have described subjects with pain and normal structure, two thirds of tendons broken by degeneration did not cause pain before reaching this [24]. The normalization of the structure is a different process from the normalization of symptoms, but not less important. Therefore when a patient overcomes the symptoms does not mean that his recovery has been total, but that a treatment plan must be continued since sometimes the pain is only the visible tip of the iceberg [3]. Therefore, not only symptoms or only structure. Not just pain or just image. The two, starting with the clinical presentation.

4. Update in the Therapeutic Approach

Let's start first with how they have been treated so far. If we enter a Physiotherapy or Traumatology consultation, tendon treatments have been based on:

4.1. From Traumatology

- *Infiltrations of corticosteroids or, in cases of poor evolution, debridement or "raking" surgery on the tendon:* with respect to infiltrations, these substances produce pain reduction, but not an improvement at a structural level since they are inhibitors of collagen synthesis [25, 26]. This means, they can produce a decrease in symptoms in the short term due to its analgesic effect, but according

to what Cook has explained to us, in the battle degeneration/healing these substances can contribute to the degeneration of the tendon [27]. Literature reflected this briefly with case studies [28], and more recently studies on epicondylalgia show how 72% of cases relapsed in the first year compared to 7% in the control group [29]. Likewise, this is like the non-existence of studies that say that throwing yourself from the plane with a parachute is more effective than throwing yourself without it, it is not proven. The same argument could be used to elaborate an opinion regarding the surgical "raking," which seeks to damage the tendon to produce an inflammation that will heal it. Perhaps in a degenerative tendinopathy, but in a reactive one?

- *Casting:* for a drastic moderation of the tendon load. Understanding the mecanostransduction, not only it will not help the tendon to heal because of the lack of loading at the same threshold to stimulate this process, but it will also produce a drastic drop in this threshold [30], predisposing the tendon to structural weakening that makes it more susceptible even to the load [9, 31].
- *Compressive orthoses:* like the epicondylar or patellar strap. This approach is shared with Physiotherapists who use bandages to create the same effect. The reasoning to place this type of device is that the tendon is overloaded by the tensile force exerted on the muscular belly and if we exercise, therefore, a compression we will not let that force overstretches the tendon. If our tendinopathy comes by tensile forces, these techniques can be beneficial, but if we consider also the compressive forces we may be, again, contributing to aggravate the pathology [32].

4.2. From Physiotherapy

- *Passive therapies:* Manual techniques, such as Cyriax friction massage, or other physical agents have been used classically for tendinopathies. Currently the bibliography shows no significant

effects of ultrasound [33], no consensus on the use of shock waves [34], slight efficacy of the laser [35] and slight positive effect of manual therapy [36, 37], although with low level of evidence. That is, variable results on the efficacy of these techniques without conclusive results [12]. Apart from the techniques applied directly in the tendon, in this section we should mention those applied in the muscle since, as we have commented from the beginning, both structures are part of a single functional unit. There are studies that show no effectiveness of dry needling in tendinopathies [38] and this makes a lot of sense because, as we see in the Continuous Model, tendinopathy implies much more, starting with the tendinous tissue itself. But clinically, we must bear in mind that the presence of myofascial trigger points on the musculature is a continuous longitudinal load on the tendon.

- *Invasive techniques:* Some authors have proposed to re injure the tendon with some treatments such as needles or surgical raking, aggressive therapy for soft tissue, and other emerging techniques in tendinopathies. The most known has been the intratissular percutaneous electrolysis or EPI ©, which consists in conducting a continuous current, and therefore with a chemical component), through the tendon utilizing a needle. To be precise, it must be guided by ultrasound. The only studies carried out on these techniques are case studies and, therefore, it has a very low level of evidence. More evidence is needed before to obtain conclusions of this type of invasive techniques, but having the information of the Continuum Model, they could have some use in degenerative tendinopathies, where acellular areas would be destroyed and an inflammation would be caused, but perhaps never in reactive tendinopathies because in this phase producing more damage would lead to failure in the attempt to heal. A reasoning like the one we mentioned in the case of "raking" surgeries.
- *Eccentric exercise:* In 1998 a Swedish surgeon, Alfredson, suffered a tendinopathy in the Achilles and wanted to have surgery. Since the waiting list was long, he tried to tear it so that he could be operated

> on urgently. For this, he put a backpack on his back with very heavy loads and began to make eccentricexercises, and to his surprise he did not tear it but he improved. That is why he started to investigate. Although Stanish et al. [2] already gave the first notions about the relationship between eccentric exercise and tendinopathies in 1986. It is to him that we owe the idea of eccentric exercise for tendinopathies [38] that we have always assimilated in universities but, above all and more importantly, it is the first approach of mechanotherapy in the treatment model.

Manual therapy will help us but, if we have understood what has been explained so far, we must keep in mind that in a problem that comes from loading, something must be done with loading as well. So we will talk about the technique that is key to solving tendinopathies: mechanotherapy.

Initially, we might think that what we have to do is remove all kinds of loads so that the regeneration will win over the destruction. But we know that this brings damage from the other extreme: the physiological threshold goes down even more and with less threshold the tendon is hurt. So you have to load to stimulate the collagen formation... but when, how much and what kind?

After Alfredson, who remained a protagonist for life as a precursor, there have been more specialists who began to investigate this load regulation. Silbernagel (2001, 2007 and 2011) [40, 41, 42] established a protocol based on concentric and eccentric exercise performed daily and progressing first in speed and then in loading. Kongsgaard (2009) proposed the Heavy Slow Resistant training (HST) [43], concentric and eccentric with high loads. If you look, both broke the trend of isolated eccentric exercise. After all, is the tendon used alone in eccentric in our daily activity? Absolutely not, and we must adapt it to everything.

After that, the studies of Naugle in 2012 [44] and Rio in 2015 [45] analyzed the isometric exercise, noting that it has a potent analgesic effect in tendon pain. Contractions of long duration and of low to moderate intensity (25-50% of the maximum voluntary contraction) produce a reduction of the pain and a cortical inhibition. Cook & Purdam also discussed the

isometrics in 2013, proposing the repetition of these contractions several times a day in contractions of 40/60 seconds x 4/5 repetitions to reduce pain and, very importantly, to maintain the capacity of the tendon since this type of work also produces changes in tendon stiffness [9, 46].

But, despite these research, the answer to these three questions is still as the diagnosis: clinical. We know about the pathophysiology and we know that exercise, compared to manual therapy techniques, is the most effective treatment. But today the evidence has not shown us which protocol model integrating all this information is morebeneficial. Mallarias reviewed the literature on exercise effectiveness in Achilles and patellar tendinopathy in 2013 [47], finding numerous articles with methodological errors in their design.

The evaluation of the approaches also depends on whether it is aimed at pain, structure or function, which as we have cited these three variables are not established in a direct relationship. If we direct it towards pain, we have evidence about the effectiveness of the isometrics as we have already seen. If we direct it towards the structure, studies show that reversible degeneration is limited. Although the continuum suggest the use of treatments to stimulate regeneration, interventions have shown that it doesn't necessarily has been achieved. Therefore, regarding the structure, the real objective is to optimize the adaptation of the tendon as a whole. That is, increase the capacity of the non-degenerated tendon area, explained in the metaphor "treat the doughnut, not the hole" [1]. Regarding the function, we hardly have any evidence because studies have currently focused on the other two variables.

In summary, at the treatment level, the evidence to date cannot reveal any conclusive exercise protocol at the moment.

Therefore, the proposals are to attempt to treat the person we have in front according to the findings in the evaluation and with the information that we know. So, if we take the information that we have to the phases described, the approach would be similar to:

4.3. Phase 1: Load Modulation (Pain Reduction)

The first thing will be to modify the overload that has produced it to establish the conditions under which healing is possible. For that we look for a load that does not exceed the current threshold, but aware that is not too low that does not reach it to prevent it from falling.

Therefore, stopping completely the sporting activity may not be necessary, although it may need to be moderated to a point where it does not exceed this threshold. We should remember that this also comes when an individual stops an activity completely and then resumes it. In the study conducted by Silbernagel [56] in volleyball players, those who performed the treatment stopping the activity did not improve more than those who moderated it.

And knowing that the isometric exercise has an analgesic effect, this work is presented as the most appropriate tool to usein the clinic: isometric contractions of 40/60 seconds x 4/5 repetitions per day according to irritability. This dose will be adapted in cases when wefind tendon irritability upon reevaluation, that is, the pain that occurs after having performed an activity, the most representative being the morning stiffness that the patient experiences the next day.

The isometrics, in addition to starting the treatment, also serve as confirmation of our diagnosis if they achieve a positive effect in reducing pain.

In addition to regulating the longitudinal loads we will also have to be attentive to reduce also the compressive ones: to check the footwear, positions and etc. The stretches, normally indicated for the muscular part, can impose a load of both types to the tendon and increase its reactivity. Stretching is not good for any symptom, and this is one of those situations.

In summary: reduce factors that add compressive or tensile load, moderate frequency and volume of exercise, isometric exercise.

4.4. Phase 2: Increase Strength

The modulation of the load allows the tendon to begin healing. Once recovered we have to restore the threshold it had before it was injured, so

we start progressive loading. When we talk about strength, we talk about muscle. But in the end, it is the tendon that transmits it, so the training will be the same for both. Therefore, it is not surprising that strength deficits are a predictor of tendinopathy [48].

Once the pain has disappeared we want to restore tolerance to the load. Let that tendon go back to what it was. And for this there are several options although, as we have said, we do not have a proven recipe.

At the end of the 90s the eccentrics of Alfredson were the standard for this. But now it is known that we can not leave aside the concentric, since then both types are used in our daily life. The proposed options are the Silbernagel protocol or the Heavy Slow Resistance (HSR) of Koongsgaard (Table 1):

Table 1. Approaches for strength by different authors

	Type of contraction	Load	Series	Frequency
Alfredson	Eccentric	Body weight initially increases as symptoms reduce	3-15	2 x day x 12 weeks
Silbernagel	Concentric/ Eccentric	Initially body weight, increases according to capacity	Several	Daily 12 weeks to 6 months
Koongsgaard (HSR)	Concentric/ Eccentrric	15-6 *repetition maximum* (RM) progressive in time	4-15	3x week x 12 weeks

Since our objective is to increase strength, Alfredson's protocol would be the first one to rule out by isolating only one type of contraction. One could choose one or the other of the other two proposed. The HSR has the disadvantage that it needs gym machines, and many times either they are not accessible to the patient or they represent an impediment in the adherence to the treatment.

But speaking of strength training, and in the absence of evidence to date, the system of series and repetitions according to RM that we have always done is equally valid. The important thing is to choose well an exercise that focuses the contraction on the muscle whose tendon we want to work and propose a system of repetitions and series. It should also be ensured that it does not imply compressive loads in the tendon at the beginning of this

phase, to evolve later to positions that do produce them (evolution in tensile and also compressive loads). For example, in a heel elevation for the Achilles we would start in a journey in intermediate positions to work later closer and closer to the dorsiflexion.

Studies indicate that, under normal conditions, a tendon takes around 36-72 hours to repair its tissues after a physiological load [49, 50], so we should let 2 and 3 days pass in between sessions.

Phase 2 can also be started if the pain has not disappeared but has fallen to a minimum. Both phases would overlap with the introduction of the first isotonic exercises while continuing the pattern of isometrics to continue descending the pain.

In summary: after the pain has been overcome, the controlled loads continue to increase, passing to isotonic work.

4.5. Phase 3: Shortening/Stretching Cycles

Once these two phases are over, with the tendon already painless and its level of strength restored, the treatment would have ended if my normal activity simply involves activities such as walking. But if for example the patient performs a sport, the rehabilitation of the tendon must continue with more demanding and explosive types of work such as those that will be required in this type of activity.

That is, it would go from increasing load to increasing speed. For this phase, the plyometric exercise will be indicated, especially in the tendons of the lower limb, since its operation is based on the storage and return of energy. It evolves to this phase when, in addition to the disappearance of the symptoms, the strength and resistance have been restored.

Again, for this the evidence has not come this far. Clinically the same exercises of phase 2 can be used adding the speed component and respecting the same frequency.

In summary: we would continue the work of the tendon with loads of maximum speed and amplitude.

4.6. Phase 4: Return to Sport

And for the greatest of the possible progressions, which is what the tendon is known to require, progress will be made to the gestures proper to the sport to which the tendon will later sustain. For example, we will make trials of activities as real as possible so that afterwards he is always prepared and adapted.

To do this, the exercises must be adapted to specific gestures of each sport, always bearing in mind the word progression must be the director that marks the reincorporation.

It is recommended to treat patients initially with exercise for at least three months before considering other options [51, 52, 53].

5. TENDINOPATHIES: MOVEMENT SYNDROMES AND MOTOR CONTROL

Besides load management, there are risk factors added to this cause:

1) *Systemic factors:* the age and health status of the individual. Metabolic diseases that influence the quality of tissues, such as diabetes, dyslipidemia or hypertension, taking medications such as fluoroquinolones (antibiotic), or habits such as smoking have been described as risk factors [3, 54, 55].
2) *Kinetic chain factors:* Many patients with tendinopathy tend to move in patterns that suppose an excessive or abnormal load on their tendons [56], which may be the cause or contribution of the chronicity of these processes.

An Achilles may have been overloaded because it has been subjected to many kilometers of race, or not many kilometers but with a gluteus medius that does not control the landing and makes the hindfoot overpronated. A supraspinatus may have been overloaded because it has raised the arm many

times, or few times but the individual also has a trapeze that every time the shoulder is flexed does not raise the acromion and this tendon is taking an extra compression. The existence of a movement syndrome [57] could make each repetition support more load than it would imply in a controlled kinetic chain, and could produce an overload even with a moderate number of repetitions.

Alterations in motor patterns or lack of variability in movement strategies in reaction to the environment could lead to tendinopathy. Some studies show how jumpers with patellar tendinopathy bend the knee more in the absorption phase of the jump [58], or how the scapular dyskinesia is related to rotator cuff tendinopathies [59] and, especially and very importantly, those with less variability in movement than the healthy [60]. The variability in movement minimizes the accumulated load in a specific region and if it is reduced it can be observed in, for example, a small coefficient of variation in knee flexion angles during the landing task [61]. Non variable motor patterns imply that the corticospinal control is altered in some way, and may be due to underlying protective strategies.

In addition, just as these motor changes can cause tendinopathy, tendinopathy may also induce changes in motor control as protective strategies [62]. But whatever the beginning, these motor changes may not resolve spontaneously or normalize after the recovery of tendinopathy, making it noticeable that rehabilitation has not valued this motor estrategies, leaving the patient vulnerable to recurrence.

The current therapeutic approaches, as we have described in previous sections, aim to restore muscle and tendon properties using exercise in a variety of protocols. But it is possible that in all of them the objective of restoring motor or corticospinal control of them is lacking. They are always aimed at producing tissue adaptation, and there has been little or no focus on modulating the corticospinal control. Variations in the patterns of force exercises that introduce sound or visual variables to which to adapt contractions, such as the use of a metronome in force work, for example, where the individual will contract concentrically, isometrically or eccentrically, following the sound imposed or following a visual signal, have shown that it is already capable of producing changes in excitability [63, 6, 65].

The evaluation of the movement or kinetic chain is something that we must perform in all injuries, since the restoration of movement is the ultimate goal of every physiotherapist. Coombes proposes that a complete approach for tendinopathies should include the treatment of the tendon, the syndromes of movement disorders and the nervous system [66]. So this point we should not forget either in the case of tendinopathies, because if this factor is involved we must assess it and introduce it in the treatment plan for its complete resolution.

And if we talk about movement, and within the framework of the evolution of a biomedical system to the biopsychosocial model, the management of psychosocial factors such as the belief systems and expectations of patients in the management of tendinopathies will be essential. How they affect the prognosis of tendinopathy also needs to be explored and investigated [67].

6. MAJOR MESSAGES

- Currently, tendon injuries are classified as "tendinopathies," since the Cook and Purdam Continuous Model is accepted as a pathophysiological model that explains the evolution of these injuries from the reactive phase to the degenerative phase. Within this model, inflammatory and degenerative coexist, and the integration of all of them continues to be investigated. To date, the relationship between structure, pain and function has not been explained by any of them and future studies are necessary.
- The diagnosis of tendinopathies is, to this day, clinical. Fulfilling the three rules of pain localized with the tip of the finger pain, pain On/Off and changes in the history of loads. This last point is critical as a cause of tendinopathy since it is a tissue with a slow metabolic rate and, therefore, with a slow adaptation to the load. The imaging tests will provide us with information about the structure, but they do not serve in themselves to issue a diagnosis because of the direct relationship between structure, pain and function.

- No passive therapy is as effective for tendinopathies as mechanotherapy, i.e., exercise. As a fundamental model in Physiotherapy like the use of the isometrics in acute phases to help maintain a joint and the concentric-eccentric after to rebuild the muscle mass, it has also been shown to be valid for tendon, and several authors have studied proposals for therapeutic approaches to the prescription of loading. This also implies a connotation for which, starting with the physiotherapists themselves, we change our mentality: not everything is to lay hands on the patient, or "hands-on." Many processes require a "hands off" approach or treatments in which we do not need to do it. The mechanotherapy, treatment, an excellent "hands off" approach, implies that we make the patient aware and participant in his own recovery process. If the treatment for tendinopathies continues to be l massage, ultrasound and ice, we have totally missed the boat. Let's load it.
- Around the non-progression of loads as a cause of tendinopathy, there are risk factors such as systemic and kinetic chain ones. The existence of syndromes of movement impairments, alterations in motor control, changes in cortical excitability or little kinetic variability can be both perpetuating and consequences of tendinopathies. Future research should include these aspects in the proposals for treatment approaches with loading exercises.
- As in the management of any injury in the psychosocial approach, these aspects, such as the beliefs and behaviors of the patient, should be investigated in tendinopathies since there are currently no investigations in this regard.

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BIOGRAPHICAL SKETCHES

Ana Gacimartín-García

Affiliation: FREMAP Working Accidents Insurance Company, Madrid, Spain

Education: PT, MSc

Research and Professional Experience: FREMAP since 2008. Private practice at Fisiomon since 2014. Rio 2016 Olympic Games – Field Hockey Stadium medical services. Researching at musculoskeletal injuries at FREMAP.

Honors: III Physiotherapy Training Sessions of the Fuenlabrada Hospital. Mention of the scientific committee for best oral communication: "Detection and prevention of injury risks after biomechanical analysis of the sport gesture in field hockey players"
http://www.jornadasdefisioterapia.info/jornadas/2013/finish/6-3a-jornada/70-comunicacion-03-deteccion-y-prevencion-de- riesgos-lesivos-tras-analisis-biomecanico-del-gesto-deportivo-en-jugadores-de-hockey-hierba

Luis F. Prato

Affiliation: Lakeshore Bone and Joint Institute, Portage, IN, US

Education: Bachelor in Science of Physical Therapy

Research and Professional Experience: University of Valparaiso Human Movement Laboratory. Sports Physical Therapy Coordinator at Lakeshore Bone and Joint Insititute. Editorial Board member of Sports Medicine Open.

Honors: Board Certified Orthopedic Clinical Specialist, Board Certified Sports Clinical Specialist, Certified Strength and Conditioning Specialists

Luis Espejo-Antúnez

Affiliation: Department of Medical-Surgical Therapy, Medicine Faculty, Extremadura University, Badajoz, Spain

Education: PT, MSc, PhD

Research and Professional Experience: Teaching and research (Physiotherapy Area). Profesor Contratado Doctor. Member of Research Group CTS 947: Salud y Actividad Física para la Calidad de Vida, Sistema de Información Científica de Andalucía, Seville (Spain).

Honors:

- Accredited by the National Agency for Quality Assessment and Accreditation (ANECA) in figures Associate Professor, Assistant Professor Doctor and Professor Hired Doctor.
- Teacher evaluation (DOCENTIA program) as professor at the University of Extremadura: outstanding teacher (mention of teaching excellence).
- III Physiotherapy Training Sessions of the Fuenlabrada Hospital. Mention of the scientific committee for best oral communication: "Detection and prevention of injury risks after biomechanical analysis of the sport gesture in field hockey players"
- http://www.jornadasdefisioterapia.info/jornadas/2013/finish/6-3a-jornada/70-comunicacion-03-deteccion-y-prevencion-de-riesgos-lesivos-tras-analisis-biomecanico-del-gesto-deportivo-en-jugadores-de-hockey-hierba

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Chapter 3

INTRATHECAL MIDAZOLAM AS AN ANALGESIC IN CHRONIC BACK PAIN

Jan Procházka*, MD, PhD
Department of Anesthesiology, Perioperative Medicine
and Intensive Care, J. E. Purkyně University, Masaryk Hospital,
Ústí nad Labem, Czech Republic

ABSTRACT

Background. The antinociceptive effect of intrathecal midazolam is based on its affecting spinal gamma-amino butyric acid receptors.

Objective. To evaluate pain relief in patients with chronic low back pain and failed back surgery syndrome after a single-shot intrathecal administration of midazolam.

Design. A prospective, open-label study.

Outcome Measures. The analgesic effect was determined using a patient questionnaire during subsequent visits to the pain therapy service. We classified a pain reduction of 50% or more as a positive outcome with improvement in quality of life and functional condition.

* Corresponding Author E-mail: jan.prochazka@yahoo.com; jan.prochazka@kzcr.eu.

Results. Between 1995 and 2017 we performed 748 administrations: 319 administrations in 63 male patients and 429 administrations in 85 female patients. We performed 93 administrations for chronic low back pain and 655 administrations for failed back surgery syndrome. The average age of our patients was 51.8 years (range 28 to 86). The dose administered ranged from 2 to 5 mg of midazolam. The analgesic effect lasted 9 weeks on average, ranging from 1 week to 3 years; median of 5 weeks. In 65% of patients we achieved pain relief lasting 4 weeks or longer; in 11%, the administration provided no analgesic effect at all. The incidence of side effects (drowsiness, nausea, headache, or transient worsening of complaints) was rather low.

Conclusion. Intrathecal midazolam is a useful supplement to standard analgesic therapy with opioids, non-opioids, or spinal steroids.

Keywords: intrathecal midazolam, failed back surgery syndrome, chronic low back pain, spinal analgesia, off-label

1. INTRODUCTION

Spinal opioids are widely used for pain relief in acute or chronic pain. Patients indicated for spinal opioids usually require a sophisticated device for continuous administration. However, spinal opioids are not suitable for all patients. In cases of a weak analgesic effect of spinal steroids there are a very few alternatives. Intrathecal midazolam is one such alternative for spinal analgesia.

Chronic low back pain (LBP) and failed back surgery syndrome (FBSS) are serious medical conditions with a significant health, social, and economic impact. Causes of chronic LBP and FBSS are usually multifactorial. Low back pain can persist in patients who have already undergone surgery and are not indicated for another surgery due to an elevated risk of failure. Furthermore, pain can persist after spinal surgery despite the absence of any correlates found by radiographic examinations. These patients fall into the FBSS category and become candidates for comprehensive pain management in pain therapy centers, which provide various interventional therapies. Therapeutic interventions should be distinguished according to the type of pain, which can be either somatic or

radicular (Manchikanti 2009), while pain etiology can be somatic, neuropathic, or mixed, especially in the case of FBSS. Spinal steroids administered via a caudal or intervertebral approach, preferably under fluoroscopic control, are widely used in patients with chronic LBP or FBSS (Conn et al. 2009; Parr et al. 2009). However, there are only a few simple, minimally invasive and low-cost options to treat those patients in whom spinal steroids provide minimal analgesic effect. These patients are subsequently destined for spinal cord stimulation or implantable drug delivery systems – both relatively costly methods. Another option for pain treatment is intrathecal (IT) administration of midazolam (Goodchild et al. 1997).

Midazolam was the first clinically employed water soluble benzodiazepine and is commonly used in anesthesia and intensive care. Unfortunately, a preservative free, commercially available formulation is not available in the United States. This formulation is applicable for anesthesia or long-term sedation, but not for intrathecal administration and should not be used intrathecally in patients with noncancer pain unless they are at end of their life (Deer et al. 2010).

The analgesic effect of spinal midazolam has been known since 1980 and is based on influencing spinal gamma amino butyric acid ($GABA_A$) receptors (Niv et al. 1983; Munro et al. 2013). In 1986, it was demonstrated that there is a high density of $GABA_A$ receptors in lamina II of the dorsal horn of human spinal cord, possibly explaining the pain modulation effect of midazolam (Zencirci, 2014). Midazolam binds with these receptors and acts as an agonist (Ho and Ismail, 2008). It decreases excitatory synaptic transmission at the benzodiazepine/$GABA_A$ receptor in the interneuron, decreases excitability of spinal dorsal horn neurons and increases duration and amplitude of GABAergic synaptic current by acting on benzodiazepine/$GABA_A$ receptors in substantia gelatinosa neurons, subsequently involving phasic and particularly tonic neuronal plasticity. This is the main pathway for intrathecal midazolam induction of segmental analgesia. Midazolam also acts on sigma opioid receptors in the spinal cord and on kappa opioid receptors (Mody, 2005; Nishiyama, 2015).

Functional plasticity of inhibitory synapses plays an important role in adaptation of neural excitability in the central nervous system (CNS). The neurotransmitter gamma aminobutyric acid (GABA) mediates most of neural inhibition in the brain by acting through GABAA receptors. These receptors are key elements involved in establishing inhibitory tone of neurons in the brain. Activation of the $GABA_A$ receptor leads to an influx of chloride ions and to membrane hyperpolarization. Thus, 16 subunits of $GABA_A$ receptor have been identified: 6 α subunits, 3 β subunits, 3 γ subunits, and the δ, ε, θ and π subunits. Benzodiazepines interact with subunit combinations of (α1)2, (β2)2 and γ2. Other allosteric binding sites are the barbiturate site, the site of general anesthetics (both intravenous – etomidate or propofol, and inhalational – halothane) and the site for channel blocking agents such as picrotoxin. Ethanol also interacts with extra synaptic $GABA_A$ receptors depending on its concentration in the brain (Froestl, 2011; Lüscher and Keller, 2004; Munro et al. 2013; Sieghart, 2006).

The great advantage of intrathecal midazolam is the possibility of a single shot administration (Serrao et al. 1992). The purpose of this retrospective open study was to evaluate the effectiveness of single shot treatment with intrathecal midazolam in patients with chronic low back pain (LBP) and failed back surgery syndrome (FBSS).

2. Material and Methods

In our pain relief center, we developed a three-stage algorithm for patients with chronic LBP or FBSS (Figure 1). In the first step there are local interventions (trigger point injections, sacroiliac joint injections, or facet joint injections). In cases where the analgesic effect is not sufficient, we proceed to the second step – applying epidural steroids through a lumbar or caudal approach. If this second interventional step is not sufficient either, we proceed to the IT administration of midazolam as a third step. All these interventions are used as a supplement to oral opioids or non-opioid analgesics (Procházka et al. 2011).

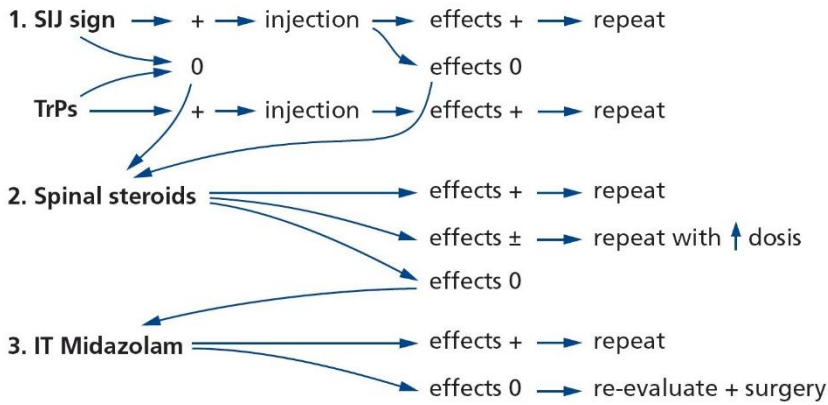


Figure 1. Our three-stage algorithm for interventional therapy in low back pain.
SIJ sign = sacroiliac joint pain; TrPs = myofascial trigger points.

After obtaining written informed consents, midazolam was administered as a single-shot injection in outpatients. We strictly used only preservative-free midazolam (Dormicum F. Hoffmann-La Roche Ltd., Basel, Switzerland, or Midazolam Torrex, Torrex Chiesi Pharma GmbH, Vienna, Austria), and the dose of midazolam was dissolved in 5% glucose or in normal saline solution up to 3 mL in volume. All administrations were performed with the patient in a recumbent position after local anesthesia of puncture site, with a small-gauge spinal needle (initially 22G, but in 1997 we switched to 25G). After administration, patients remained at bed rest for approximately 3 hours for observation before they are released. This serves as a prevention of post-dural puncture headache (PDPH) and enables monitoring of vital signs following the procedure.

The initial dose was always 2 mg of midazolam, and according to the analgesic effect achieved, we could increase the dose up to 5 mg in a single injection. These doses correspond to 0.02 – 0.06 mg/kg of midazolam. A 5 mg dose of midazolam is considered to be a ceiling single shot dose for chronic non-malignant pain. Time intervals between subsequent administrations were at least 4 weeks.

When we aspirated the cerebrospinal fluid back into the syringe filled with midazolam solution to confirm correct needle position in the subarachnoid space, we observed a slight turbidity of the solution. This

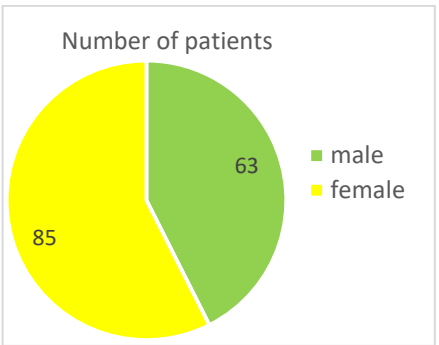
turbidity in otherwise clear solution was most likely caused by precipitation of cerebrospinal fluid (CSF) proteins due to low pH of the solution (pH = 3.3). This level of pH is necessary for lipid solubility when midazolam is exposed to physiologic pH. This lipophilicity is responsible for its rapid CNS effect (Zencirci 2014).

The analgesic effect was evaluated during subsequent patient visits, specifically analysis of subjective pain relief achieved for each patient. Intervals between administration and a follow-up visit were determined by the patient's individual needs. Follow-up visits came at intervals of 4 weeks to 9 years. In some cases, patients ceased the treatment or had their analgesic doses transiently decreased (we describe these periods as "analgesic holidays"). We considered at least a 50% pain reduction with improved quality of life (QoL) and improved functional condition with focus on the overall feeling, quality of sleep, housework, or hobbies capability, etc. to be a positive outcome (in accordance with Moore et al. 2010).

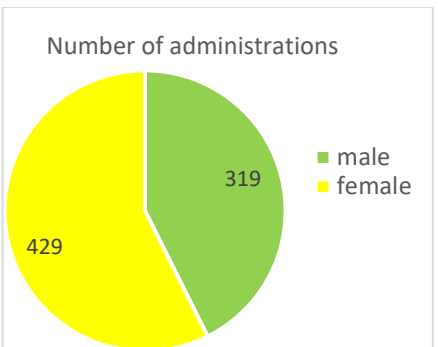
After midazolam administration, we evaluated the quality of sedation using a four-point scale as follows: 0 = wide awake and alert; 1 = at times drowsy but easily aroused; 2 = somnolent but easily aroused; and 3 = somnolent and difficult to arouse (Yegin et al. 2004). Some early side effects were registered during bedside visits; late side effects were recorded during subsequent visits of the patient to the pain therapy service.

3. RESULTS

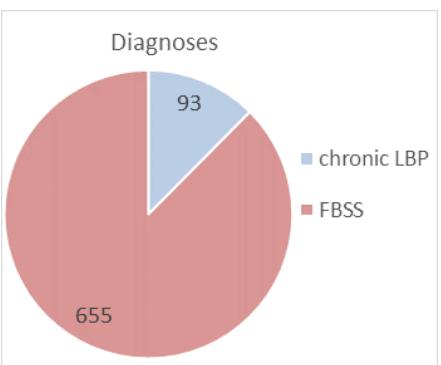
Between 1995 and 2017, we performed 748 administrations in our department: 319 administrations in 63 male patients and 429 administrations in 85 female patients. We performed 93 administrations in patients with chronic LBP and 655 administrations in cases of FBSS (for summary of the demographic data see Graph 1 – Number of patients, Graph 2 – Number of administrations and Graph 3 - Diagnoses). The average age of our patients at the time of administration was 52 years (range 28 to 86). The dose administered ranged from 2 to 5 mg of midazolam (Table 1).



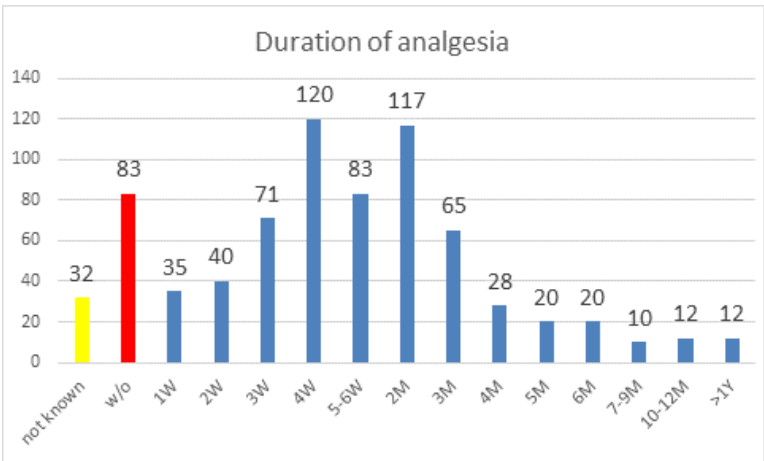
Graph 1. Number of patients.



Graph 2. Number of administrations.



Graph 3. Diagnoses indicated for IT midazolam.



Graph 4. Duration of achieved analgesia after a single intrathecal administration.

Table 1. Administered dose of midazolam

Dose of midazolam	Number of administrations
2 mg	211
3 mg	184
4 mg	154
5 mg	199

The duration of pain relief is shown in Graph 4. After 83 administrations (11%), we observed either no pain relief, only weak relief (less than 50% pain reduction), or too short duration of pain relief (only several days). In 32 cases (4%), we have no information about the extent of analgesia achieved. Average pain relief duration achieved after a single spinal injection was 9 weeks, median 5 weeks; in 65% of the cases we achieved a significant pain reduction lasting more than 4 weeks.

The incidence of side effects was low. The most common side effects were drowsiness in 1 to 3 points on a four-point scale (approximately 31% administrations), headache (4%), nausea (1%), and a transient worsening of pain (7%). We did not see any clinical signs of neurotoxicity (bladder or bowel dysfunction or new radiculopathy) following midazolam administration, even though we performed more than 10 intrathecal administra-

tions of midazolam (up to 57 for twelve years) in 16 cases. Drowsiness levels vary individually among patients; as well as in the same patient based on the dose.

4. DISCUSSION

4.1. Theoretic Background

Systemic analgesics are effective in most patients with chronic pain; however, in some cases their effect is minimal. Pain relief in these patients can be achieved via spinal analgesia. This approach usually requires continuous spinal infusion via an implantable or external pump in postoperative pain management of FBSS or chronic LBP therapy. In contrast to this tactic, we employ only a single injection administered without any catheter or pump system.

When deciding to apply spinal analgesics, we must balance between risks (neurotoxicity, side effects of the drug) and expected benefits (pain relief, quality of life) (Lavand'homme 2006). When introducing a new method for spinal analgesia, one must meet the following criteria: the analgesic efficacy must be confirmed by experimental data and clinical experience, the method must be safe and well-tolerated by patients, it must be easy to perform, and the costs of the method should be reasonable.

Local anesthetic agents and opioids commonly used in spinal anesthesia and analgesia provide only a very limited duration of effect. To maximize the duration of anesthesia or analgesia many adjuvants have been tried. However, intrathecal opioids are associated with many dose-related adverse effects, such as respiratory depression, nausea, vomiting, urinary retention, pruritus or sedation. Therefore, the use of other agents – non-opioids such as ketamine, clonidine, neostigmine, midazolam or ziconotide – have become popular adjunct for post-operative or long-term analgesia (Zencirci, 2014, Nishiyama, 2015).

Intrathecal midazolam can meet many of the requirements for an ideal spinal analgesic: its analgesic properties have been confirmed in several

studies; the method has a low incidence of side effects and the patients solicit additional administrations; it is very simple – only a single-shot administration in outpatients not requiring any catheters or pumps; and finally, the costs of this method are low.

Spinal $GABA_A$ receptors play a significant role in antinociception. Midazolam is a water-soluble benzodiazepine widely used for sedation in intensive care units or in the operating room due to its sedative, anxiolytic and amnestic effects. Its spinal antinociceptive mechanism can be explained as a reduction of excitatory synaptic transmission by acting on the gamma-amino butyric acid ($GABA_A$) receptor in interneurons, leading to a decrease in the excitability of spinal dorsal horn neurons (Yaksh et al. 2004; Kohno et al. 2006). Moreover, GABAergic modulation could be involved in antinociceptive action of opioids and cannabinoid systems in periaqueductal gray matter (PAG) and rostral ventromedial medulla (RVM) (Lau and Vaughan, 2014).

In our country, midazolam is supplied as a preservative free solution with pH value between 3.0 and 4.0 (buffered with hydrochloride acid alone or hydrochloride acid and sodium hydroxide). This level of pH is necessary for lipid solubility when exposing midazolam to physiologic pH. The lipophilicity of midazolam is responsible for its rapid CNS effect (Zencirci 2014). In 1998, Nishiyama et al. studied effects of adding midazolam and bupivacaine to human cerebrospinal fluid (CSF) and this solution was examined for any changes of pH and a reduction of transparency of the solution. Adding midazolam or bupivacaine resulted in decreased pH and cerebrospinal transparency. However, midazolam in saline solution neither decreased the pH bellow 7.0 nor reduced transparency. These results showed that therapeutic doses of intrathecal or epidural midazolam were not neurotoxic (Nishiyama et al. 1998 b). We observe a slight turbidity in otherwise clear solution of midazolam, when aspirating cerebrospinal fluid back into the syringe during midazolam administrating into intrathecal space. However, this turbidity can only be seen on the small volume syringe and may be not significant when dissolving midazolam within the entire cerebrospinal compartment. Moreover, this turbidity can be useful for us in detecting intrathecal space.

4.2. Clinical Studies

The analgesic effect of intrathecally administered midazolam has been demonstrated in several animal studies, which were later followed up by clinical studies. Intrathecal midazolam was most often used as a supplement together with an opioid and local anesthetic in postoperative analgesia. Several studies discussed analgesia after a Cesarean delivery. A dose of 2 mg of intrathecal midazolam shortens the onset of spinal analgesia, moderately extends postoperative analgesia when used as an adjunct to bupivacaine and decreases postoperative nausea and vomiting (Prakash et al. 2016; Abdollahpour et al. 2015). Intrathecal midazolam appears to be an effective analgesic technique for abdominal surgery. This technique achieved excellent analgesia with low requirements for other analgesic interventions (Duncan et al. 2007). In another study, Yegin et al. (2004) demonstrated that intrathecal midazolam combined with intrathecal bupivacaine bring about longer and more effective analgesia when compared to sole bupivacaine in patients undergoing perianal surgery. This use as a single dose is considered safe, however may result in increased sedation. Iranian authors used intrathecal midazolam in labor pain therapy as an adjunct to sufentanil with no significant adverse effects. They concluded that intrathecal midazolam could be an appropriate alternative to parenteral or epidural analgesia in small hospital settings. Improved or longer lasting analgesia was achieved using a continuous IT infusion of midazolam in combination with an opioid, local anesthetics, or clonidine, with minimal drug-related side effects (Salimi et al. 2014). Meta-analysis involving 672 patients from thirteen randomized controlled studies confirmed improving perioperative or peripartum analgesia and a reduction of nausea and vomiting during Caesarean delivery, when adding intrathecal midazolam to other spinal medications. A small dose of intrathecal midazolam (1 to 2.5 mg) does not increase the duration of motor blockade, the risk of respiratory depression or of short-term neurological deficits (Ho and Ismail, 2008).

Intrathecal midazolam administration can also be utilized in settings other than post-operative analgesia, such as pain management. Serrao et al. (1992) compared 2 mg intrathecal midazolam injection with 80 mg epidural

methyl prednisolone. Both treatments produced similar improvement in one-half to three-quarters of patients over a 2-month period in patterns of activity and sleep as well as in sensory and affective components of the pain. However, even though the improvement in the two groups was similar, all patients treated with epidural steroids were taking equal or higher amount of self-administered analgesic medications after their treatment, whereas one-third to one-half of the patients treated with midazolam were taking less rescue analgesic medication during the 2 months of a follow-up period. This data is consistent with our previous study published in recent years (Procházka et al. 2011). The study of Boussofara et al. (2006) published completely opposite findings. They concluded that adding midazolam to an intrathecal mixture of bupivacaine and clonidine does not potentiate postoperative analgesia after an elective lower-extremity surgery and prolongs the motor blockade.

Borg and Krijnen (1996) reported long-term intrathecal administration of midazolam and clonidine in patients suffering from refractory musculoskeletal pain. The treatment spanned over 2.5 years and they used intrathecal midazolam in doses up to 6mg/day, which showed promising results. They discovered that such high doses did not result in any neurological deficits.

Midazolam can also be administered into epidural space, however only a few studies have been performed for analyzing this procedure. Epidural midazolam induces a wide range of analgesic dermatomes, accelerates the onset of sensory block and time to peak effect and prolongs the duration of motor and sensory blocks of epidural lidocaine. In continuous epidural administration along with bupivacaine, midazolam potentiated the analgesic effect of epidural morphine, but inhibited the analgesic effect of fentanyl. These varying effects with morphine and fentanyl can be the result of their different lipophilicities. In clinical studies, improved pain relief or an extended duration of analgesia with good cardiovascular stability after the addition of epidural midazolam was confirmed particularly in postoperative analgesia after abdominal surgery in adults (Nishiyama 2015, Nishiyama et al. 1998 a).

Indications and contraindications for spinal injections and for an examination before injection in chronic pain patients are discussed in Landers (2008) with an emphasis on correlation between patient history, type of pain, neurologic findings, and the results of an imaging examination. We always attempt to proceed from simple interventions to sophisticated techniques. All our patients undergo comprehensive medical management (CMM) for chronic LBP or FBSS (non-opioids, opioids, anticonvulsives, antidepressants, minimally invasive interventions – trigger point injections or facet joint injections), with IT midazolam serving as a supplement to this therapy in the third stage of our algorithm when epidural steroids show little analgesic effect (see Figure 1). The initial dose of 2 mg of IT midazolam has been established based on a comparative study (Serrao et al. 1992) and one of the first clinical studies about the efficacy of spinal midazolam on somatic nociceptive pain (Goodchild et al., 1992).

Since 2007, the International Neuromodulation Society has built a group of experts to evaluate evidence and organize the Polyanalgesic Consensus Conferences (PACC) to guide the practice for intrathecal drug infusion systems. They have developed algorithms for intrathecal medication approaches to treat nociceptive and neuropathic pain, localized or diffuse, in patients with cancer and noncancer pain. The current PACC update considers midazolam to be merely an adjuvant for cancer or terminal condition-related pain with localized or diffuse nociceptive or neuropathic pain in the 6th Line (Deer et al. 2017). However, these algorithms are pertinent for continuous intrathecal infusion therapy, while our method only uses a single shot administration.

4.3. Neurotoxicity

The neurotoxicity of spinally administered drugs used in pain therapy is reviewed in Hodgson et al. (1999). Neurotoxicity of spinal midazolam specifically is still a controversial topic. We must distinguish between histopathological signs (neural injury, gliosis, damage of the myelin sheath, inflammatory changes), physiological signs (changes of spinal cord blood

flow, disruption of the blood–brain barrier, changes in electrophysiology), and clinical signs of neurotoxicity (pain, motor and sensory deficits, bowel and bladder dysfunction, behavioral changes). Furthermore, many preservatives, antioxidants, or excipients used in drugs applied spinally can cause neurotoxic changes in animal models even are considered safe when administered intravenously or intramuscularly (Hodgson et al. 1999; Abram, 1996).

The controversy of spinal midazolam stems from ambivalent outcomes of animal studies, as some of them observe its neurotoxicity, while others deny it. Those its neurotoxic effect reportedly administered much higher doses of midazolam or used spinal catheters (Nishiyama, 2015). Other animal studies found no difference in the amount of histopathologic or inflammatory changes between intrathecal midazolam and saline control group (Zencirci, 2014; Nishiyama 2015). A cohort human study investigating 1100 patients as well as meta-analysis of thirteen randomized controlled studies involving 672 patients did not show any neurological symptoms after intrathecal midazolam administration (Ho and Ismail, 2008; Tucker et al. 2004). In order to minimize the risk of neurotoxicity, the midazolam we used is a preservative-free solution.

4.4. Our Data

Our study spanning over 22 years demonstrated an average pain relief of 9 weeks (median 5 weeks) after single-shot midazolam administration. In 65% administrations we achieved analgesia for 4 weeks or more. In 11% we did not register any pain relief or registered relief for only a brief period. Intrathecal midazolam was used as an adjunct to a comprehensive medical management. Adverse effects seen in our study were only mild and transient. The most common side effects were drowsiness in 1 to 3 points on a four-point scale (approximately 31% administrations), headache (4%), nausea (1%), and a transient worsening of pain (7%). We did not observe any clinical signs of neurotoxicity (bladder or bowel dysfunction or new radiculopathy) following midazolam administration, despite performing

more than 10 intrathecal administrations of midazolam (up to 57) in 16 cases. Drowsiness levels vary individually among patients, as well as in the same patient based on the dose.

4.5. Off-Label Method

Intrathecal or epidural midazolam administration is an off-label method. Labeling contains essential scientific information needed for safe and effective use of a drug. It should be informative and accurate without being promotional, false, or misleading and should be based on data providing substantial evidence of safety and effectiveness (Chang et al. 2005). The term "unlicensed" or "off-label" should not be taken to imply disapproval, nor incorrect or improper use of drugs. There are several categories of off-label therapy: (1) unlicensed medicines used when no appropriate formulation of a drug is commercially available (for example: crushing tablets; opening capsules and suspending them into liquid formulation for children; drugs prepared in a pharmacy), (2) use reaching beyond conditions of the product license (for example: a dose lower or higher than recommended; drug used in children under a certain age; drug used in the indication not covered by the license; alternative route of administration; using drug against contraindications) (Conroy, 2002). Spinal (intrathecal or epidural) midazolam administration falls into the category of "alternative route of administration of licensed drug," because midazolam injections are designated only for intravenous or intramuscular administration.

Many drugs used in anesthesiologic practices are still considered to be off-label therapy, for example: sufentanil for intrathecal use; fentanyl for intrathecal or epidural use; ketamine in obstetrics or pediatric patients younger than 16 years; bupivacaine for use in patients younger than 12 years. US Food and Drug Administration (FDA) recommendations do not regulate medical practice based on knowledge of the medical literature, medical judgment and experience. Physicians are responsible for being well informed about the drugs they are using and for basing off-label use on firm scientific rationale or on sound medical advice (Chang et al. 2005).

Development of off-label therapies, including investigative or innovative methods, alternative routes or use outside patients' age limits offers an abundant source for medical progress.

CONCLUSION

The analgesic effect of intrathecal midazolam is caused by influencing the benzodiazepine/$GABA_A$ receptors in human spinal cord. It appears to be a suitable supplement to comprehensive medical management of patients with chronic LBP or FBSS who suffer somatic or neuropathic pain. Our study proved that analgesia for a duration of 4 weeks or more, following single shot intrathecal midazolam administration was achieved in 65% of cases, with a low incidence of side effects. This method could be useful when medical therapy alone or with epidural steroids have minimal effect. Neurotoxicity of spinal midazolam is still a controversial topic, with a strong recommendation that its use in clinical practice be performed using preservative-free drugs, due to the potential of neurotoxicity of its additives. The advantage of this therapeutic method is single-shot administration, which avoids introducing any catheters or utilizing any external or implantable pumps for continuous infusion therapy.

Intrathecal midazolam remains an off-label method; however, only wide clinical experience and laboratory research can convert it to an on-label status, following the example of opioids many years ago. Until then, intrathecal midazolam for pain relief should be used in strictly indicated cases. Further clinical studies and animal neurotoxicity studies are necessary.

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Chapter 4

MANAGEMENT OF INTRA-ARTICULAR DISTAL RADIUS FRACTURES WITH A DORSAL LOCKING PLATE

Yoshihiro Abe, MD, PhD and Hiromasa Wakita, MD
Department of Orthopedic Surgery, Chiba Rosai Hospital, Tatumidai-higashi, Ichihara, Japan

ABSTRACT

Introduction. Despite the frequency of distal radius fractures, a consensus has not been reached on the optimal approach to treatment. Nevertheless, the volar plate is not a panacea for all distal radius fractures. Lutsky and colleagues reported that certain pathologic fracture patterns are more appropriately stabilized using dorsal plate fixation, and these include: (1) dorsal shear fractures, (2) dorsal *die-punch* fractures, or fracture patterns in which an indirect reduction from the volar approach cannot be obtained, and (3) fractures with associated scapholunate ligament injury.

Methods. According to Lutsky's indications, we treated 38 patients were treated with open reduction internal fixation via a dorsal approach. Clinical assessments included range of motion measurements at the wrist, grip strength, the Quick Disability of the Arm, Shoulder, and Hand score, and the Gartland and Werley score.

Results. Except for wrist flexion, favorable results were obtained for both subjective and objective parameters. Complications were rare and not serious. The most common reason for choosing dorsal plating was irreducible dorsal *die-punch* fractures.

Conclusion. The treatment of displaced intra-articular distal radius fractures with a dorsally placed interlocking plate system demonstrated similar clinical results as volar locking plate. Postoperative complications were not readily observed in the patients treated with a dorsal locking plate. Certain fracture patterns are more appropriately stabilized using a dorsal plate fixation.

INTRODUCTION

Despite the frequency of distal radius fractures, a consensus has not been reached on the optimal approach to treatment. Volar plating systems have changed the ease by which fractures can be treated compared with traditional methods such as fracture specific fixation, pinning, and external fixation. Nevertheless, the volar plate is not a panacea for all distal radius fractures. Increasingly, it has been recognized that extensor and flexor tendon injury, loss of fixation, and secondary displacement can occur after volar fixed-angle plate osteosynthesis. Distal radius fractures with a complex disruption of the articular surface, and other fractures, are preferentially treated via a dorsal approach, but many surgeons have avoided dorsal plating. This preference might be attributed to the reportedly high complication rates, which specifically involve extensor tendon irritation and rupture associated with dorsal plating [1-4]. More recently, low-profile dorsal plates have been developed in an attempt to minimize these complications. Several case series have shown excellent results with these newer, low-profile plates [5-12]. Lutsky and colleagues [8] reported that certain pathologic fracture patterns are more appropriately stabilized using dorsal plate fixation, and these include: (1) dorsal shear fractures, (2) dorsal *die-punch* fractures, or fracture patterns in which an indirect reduction from the volar approach cannot be obtained, and (3) fractures with associated scapholunate ligament injury.

The aim of this study was to clarify the functional outcomes and complications of dorsal plating for the management of intra-articular distal radius fractures, with special regard to indications for dorsal plating.

MATERIALS AND METHODS

This study was designed according to and approved by the local Medical Ethics Committee. Between January 2011 and January 2014, we treated 217 wrists of 192 consecutive patients with displaced intra-articular fractures of the distal radius. Of these, 112 wrists with displaced intra-articular fractures were treated with an open reduction and internal fixation (ORIF). We included secondary displacements that occurred within 1 month after conservative treatment. The exclusion criteria included patients with previous trauma distal to their upper limbs, or those individuals who had been treated for rheumatoid arthritis or osteoarthritis at their wrist.

Based on the theory of Lutsky [8], dorsal locking plates were selected for patients with any of the following fractures: (1) dorsal shear fractures, (2) dorsal *die-punch* fractures, or fracture patterns in which an indirect reduction from the volar approach cannot be obtained, (3) fractures with associated scapholunate ligament injury, and (4) fracture patterns in which the volar margin was distal to the watershed line with significant dorsal comminution requiring bone (substitute) grafting. If one of those criteria was met, treatment with dorsal plating was selected. Therefore, all volar plates were placed proximal to the watershed line.

Of 112 patients, 38 patients (mean age, 57.7 years; 24 women, 14 men) were treated with ORIF via a dorsal approach.

In a case with severe metaphyseal comminution, scapholunate ligament injury, and/or joint depression, external fixation and/or bone grafts or substitutes were used with either volar or dorsal plate fixation, and the frequencies at which they occurred was also examined.

For postoperative management, active finger motion and forearm rotation were encouraged immediately after surgery in all cases. Except for the cases with associated scapholunate ligament injury, a short arm splint or

external fixator was removed within 2 weeks after surgery (mean 8.4 days) and then active wrist motion was started. In two patients with associated scapholunate ligament injury (Figures 1 and 2), the external fixator was removed 2 weeks after surgery and a short forearm splint was applied for 6 weeks thereafter. Intercarpal Kirschner wires were removed 8 weeks after surgery and then active wrist motion was started.

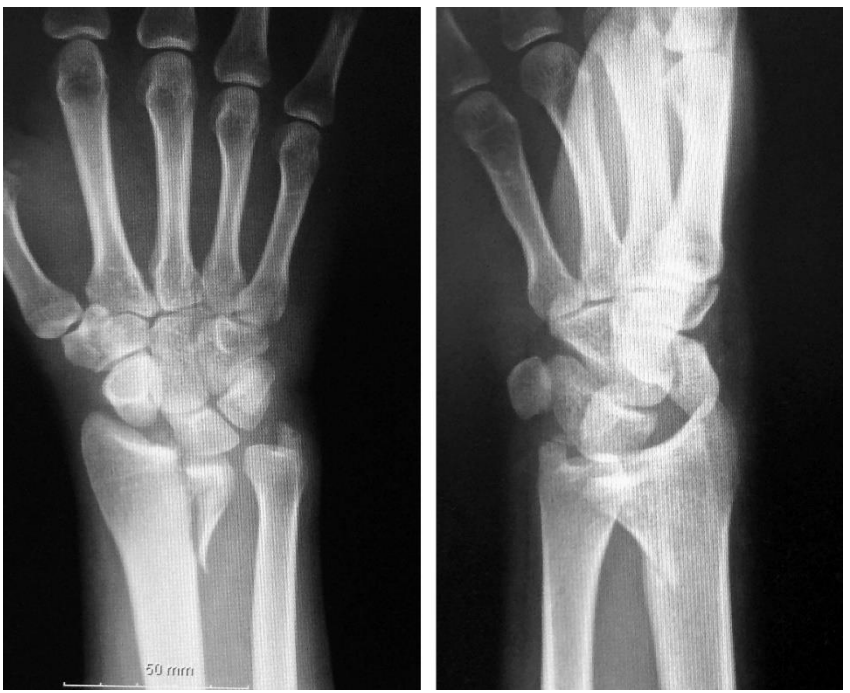

Figure 1. Posterioanterior and lateral radiographs of a 46-year-old male who sustained a fracture with an associated scapholunate ligament injury.

At our institute, implant removal surgery 8–12 months after ORIF was routinely recommended for all of the patients. Clinical data were extracted from records just before a patient's visit for implant removal in those who had consented to and underwent that surgical procedure. For the remaining patients who declined implant removal, their clinical data were extracted from their records at final follow-up (range, 8–28 months).

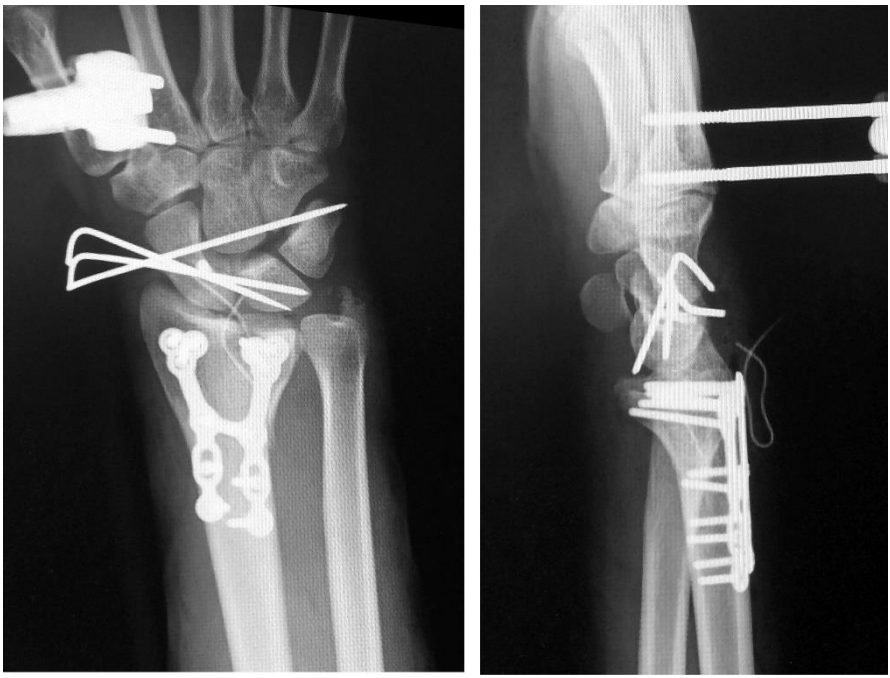

Figure 2. Posteroanterior and lateral radiographs taken after the patient was treated using internal fixation with dorsal distal plating (Aptus® 2.5 TriLock Distal Radius Plates, dorsal) and a radiocarpal external fixator. The scapholunate ligament was repaired with a suture anchor simultaneously via a dorsal approach.

Patient outcomes were evaluated using both objective and subjective parameters. Objective parameters included a physical examination of wrist range of motion, grip strength compared with the contralateral healthy wrist and radiographic evaluation. Radiographic criteria for acceptable healing were defined as a ≤ 10° dorsal tilt, ≤ 3-mm radial shortening, and ≤ 2-mm articular incongruity [13]. Subjective parameters included a patient-reported score using the Quick Disabilities of the Arm, Hand, and Shoulder (QuickDASH) questionnaire and the Gartland and Werley score [14]. Union of the fracture was defined as radiographic healing within 6 months. Finally, complications including infection, complex regional pain syndrome (CRPS), tenosynovitis or tendon rupture, compression neuropathy (carpal tunnel syndrome), unacceptable healing (> 10° dorsal tilt, > 3-mm radial shortening, and/or > 2-mm articular incongruity), and stiffness were

recorded. Carpal tunnel syndrome was determined via nerve conduction studies which showed a distal motor latency of the abductor pollicis brevis > 4.5 msec. Stiffness was defined as out of functional range of motion (ROM), which was < 40°flexion, < 40° extension, or/and a < 40° arc of radial-ulnar deviation [15].

RESULTS

Demographic data for patient cohorts is shown in Table 1.

Based on the rationale established by Lutsky for choosing dorsal plating, we treated 3 wrists (7.9%) with dorsal shear fractures, (Matrix – SmartLock®, Stryker Osteosynthesis = 3)(Figure 3a), 22 wrists (57.9%) with dorsal *die-punch* fractures, or fracture patterns in which an indirect reduction from the volar approach cannot be obtained (Synthes 2.4-mm Locking Compression Plate Distal Radius System,® dorsoradial and dorsoulnar = 22) (Figure 3b), 2 wrists (5.3%) with fractures that had an associated scapholunate ligament injury (Aptus® 2.5 TriLock distal Radius Plates, dorsal = 1(Figure 3c), Synthes 2.4-mm Locking Compression Plate Distal Radius System®, dorsoradial and dorsoulnar = 1), 11 wrists (28.9%) with fracture patterns in which the volar margin was distal to the watershed line and with significant dorsal comminution requiring bone (substitute) grafting (Matrix – SmartLock®, Stryker Osteosynthesis = 11). The most common–fracture pattern for which dorsal plating was chosen was an irreducible dorsal *die-punch* fracture. In contrast, a dorsal shear fracture with an associated scapholunate ligament injury was rarely the reason for using dorsal plating due to the relatively infrequent occurrence of that pathology (Table 2).

Supplemental bone grafts or substitutes were used in 9 of 38 (23.7%) wrists.

Clinical results were shown in Table 3.

The complications are shown in Table 4. There were no instances of nonunion, infection, and/or CRPS.

Table 1. Demographic data of 38 patients who underwent the dorsal plating for DRF

	Dorsal plate N=38
Mean age, years	53.7 ±12.7
Female/male, n (ratio)	24/14 (0.43)
Follow-up, months	13.0 ± 5.5
Bone graft or bone substitute, n	9
External fixation, n	6
Right/left hand dominance, n	11/27

[a] Data values are mean ± standard deviation unless otherwise indicated.

[b] P-values for comparisons between dorsal plating and volar plating.

* Statistically significant.

Table 2. Range of motion and outcome data for dorsal plate and volar plate groups[a]

	Dorsal plate N=38
Extension	54.2 °± 14.9
Flexion	48.3° ± 11.3
Supination	81.7° ± 7.0
Pronation	80.4° ± 7.2
Radial-ulnar deviation arc	43.7° ± 6.5
Grip strength (% of contralateral side)	61.7% ± 20.3
QuickDASH score	9.7 ± 6.3
Gartland and Werley score	2.7 ± 2.3

QuickDASH Quick Disabilities of the Arm, Hand, and Shoulder, *SD* Standard deviation.

[a] Data are means ± SD unless otherwise shown.

[b] *P*-values for comparisons between dorsal plating and volar plating.

*Statistically significant.

30 patients underwent implant removal surgery. Of these, no patients required hardware removal due to complications such as extensor tendon irritation or rupture (Figure 4). Intraoperatively, there was no evidence of

tenosynovitis or tendon damage. One patient experienced pain and paresthesia in the distribution of the superficial sensory branch of the radial nerve due to radial nerve neuropathy; neurolysis was performed with hardware removal. Although the remaining 8 patients did not undergo hardware removal, no patient experienced postsurgical-related symptoms and/or complications such as extensor tendon irritation or rupture, for example.

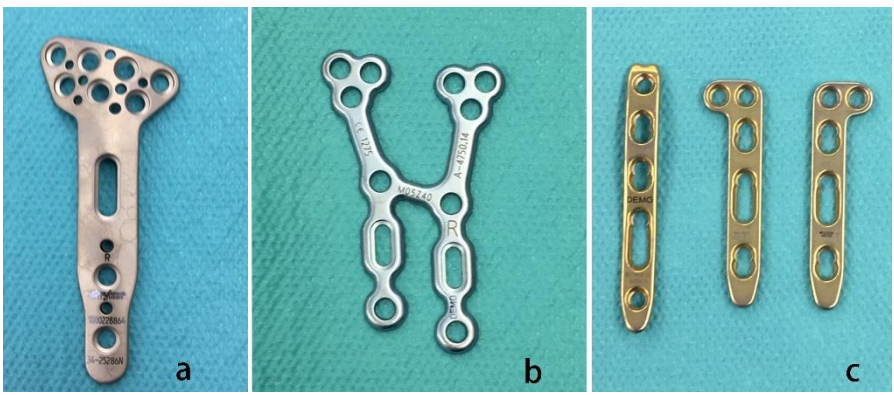

Figure 3. a. Aptus® 2.5 TriLock Distal Radius Plates, volar. b. Matrix – SmartLock®, Stryker Osteosynthesis. c. Synthes 2.4-mm Locking Compression Plate Distal Radius System®, dorsoradial and dorsoulnar.

Table 3. Fracture pattern and used implant

Fracture pattern	Implant		
	Matrix – SmartLock®	Synthes 2.4-mm Locking Compression Plate	Aptus® 2.5 TriLock
dorsal shear fracture	3	0	1
dorsal *die-punch* fracture	0	22	0
associated scapholunate ligament injury	0	1	1
distal to the watershed line and significant dorsal comminution	11	0	0

Table 4. Complications of wrist plate fixation in the dorsal plating

	Dorsal plate N=38
Tendon rupture, Extensor pollicis longus	0
Tenosynovitis, partial tendon wearing	0
Neuropathy	1 (2.6%)
Hardware failure, distal screw loosening	0
Unacceptable healing	1 (2.6%)
Stiffness	3 (7.9%)
Total complications	3 (7.9%)

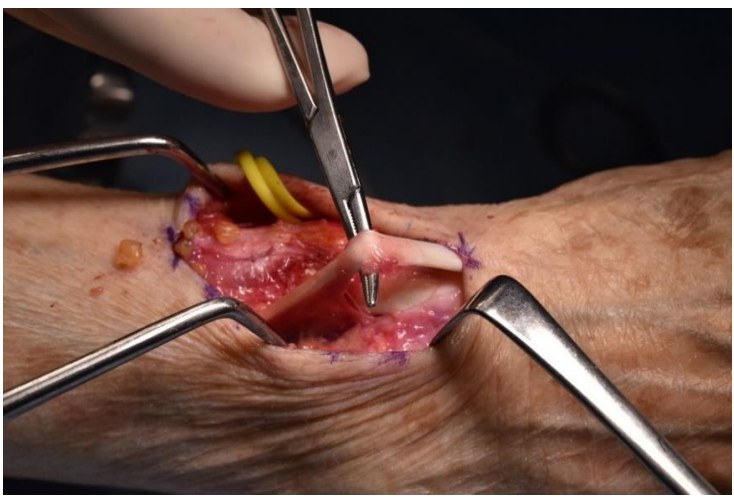

Figure 4. Intraoperative view of the case of a 69-year-old woman. The patient had implant removal surgery 10 months after open reduction and internal fixation for a distal radius fracture with dorsal plating (Matrix – SmartLock®, Stryker Osteosynthesis). There was no evidence of tenosynovitis or tendon damage.

DISCUSSION

The volar approach was initially believed to decrease the risk of tendon rupture, but there have been multiple reports of both flexor and extensor tendon irritation and rupture [16-21]. In the present series, complications

were proportionally, and no tendon rupture. In the present series, neuropathic complication was temporally neuropathy of superficial branch of the radial nerve, in which a higher rate of neuropathic complications occurred using the volar plate than dorsal plate [12].

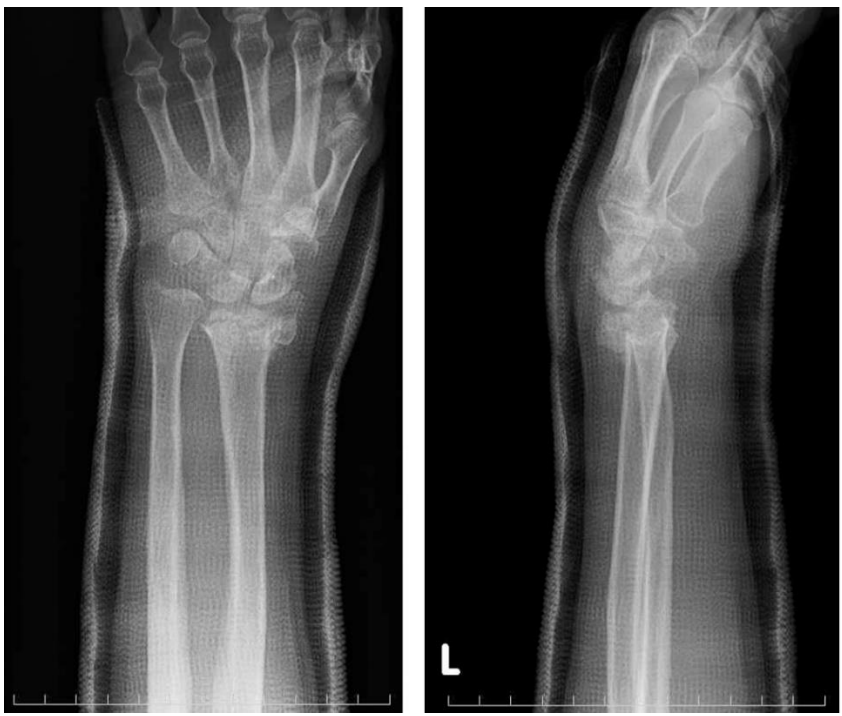

Figure 5. Posteroanterior and lateral radiographs of a 67-year-old female who sustained a fracture with secondary displacement and significant dorsal comminution in which a volar fracture margin was more distal to the watershed line.

There are two main types of dorsally placed interlocking plate systems, one is a buttress type plate and the other is a column plate. The main goal of buttress type plates is, in certain cases, to oppose dorsal displacement with the possibility of an *on the plate* reduction. The buttressing effect of the plate, if necessary, is augmented with locking screws in the distal fragments. Column plates are based on the three-column theory of Rikli and Regazzoni [10]. The two cortical bone columns of the radius injured by fractures must be reconstructed with one or several plates which may be more or less

anatomic, and must be adapted to each of these columns. Those two instruments should be complementary, because preventing recurrent displacement and restoring the anatomy are two complementary goals. Therefore, we applied buttress type plates based on the following pathologies: (1) dorsal shear fractures and (4) fracture patterns in which the volar margin is distal to the watershed line with significant dorsal comminution requiring bone (substitute) grafting (Figures 5 and 6). Dual column plates were applied for (2) irreducible dorsal *die-punch* fractures with closed manipulation (Figures 7 and 8).

Although different systems were used, unacceptable healing was not observed in the present series. This fact might validate the adequacy of our indication for dorsal plating and may relate to use of bone grafts or bone substitutes (9 of 38 wrists). This approach may have prevented radial shortening in the dorsal plated group. A recent literature review concluded that grafting may improve radiographic alignment and/or short-term outcome, but no overall influence on the final result was observed [22].

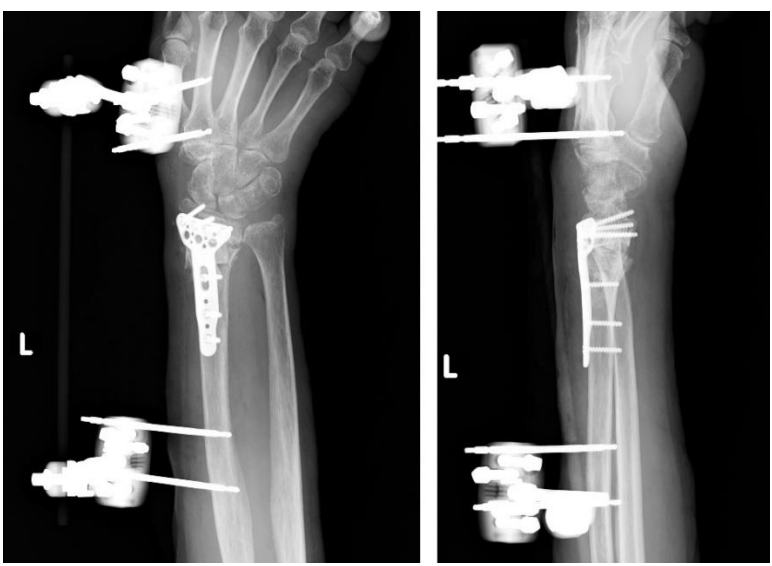

Figure 6. Immediate postoperative, posteroanterior, and lateral radiographs of the same patient after internal fixation with a dorsal plate osteosynthesis (Matrix-SmartLock®) and a radiocarpal external fixator: an iliac bone graft was applied into the resulting defect.

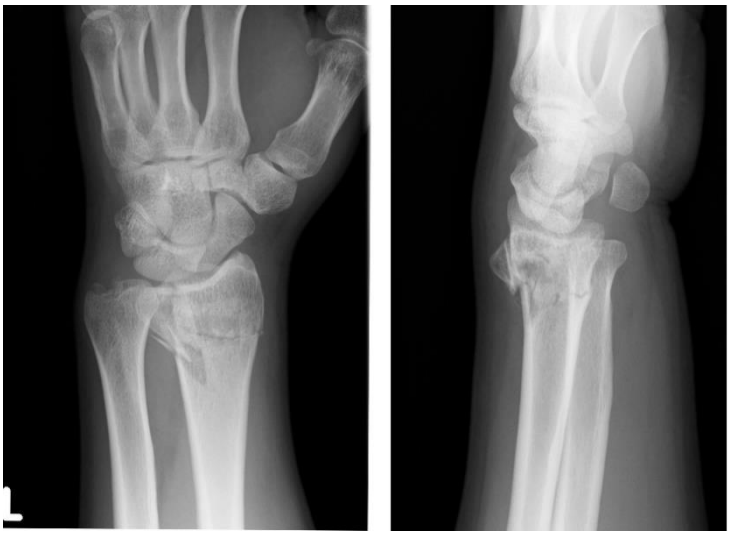

Figure 7. Posteroanterior and lateral radiographs of a 46-year-old male who sustained dorsal die-punch fractures.

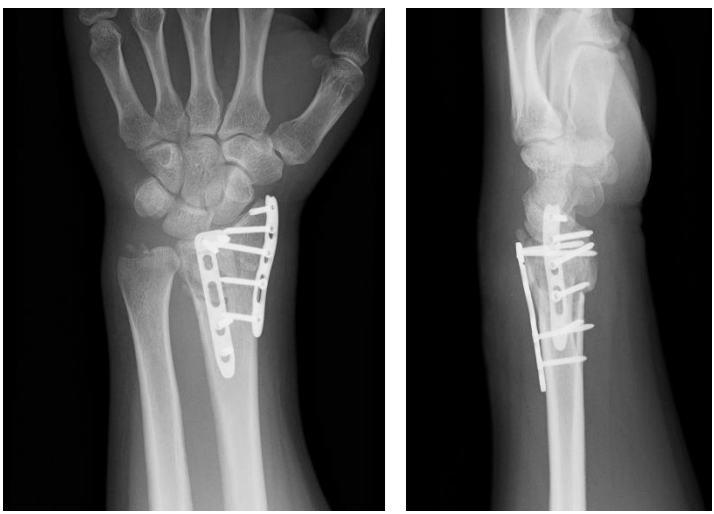

Figure 8. Immediate postoperative, posteroanterior, and lateral radiographs of the same patient after internal fixation with column plates (Synthes 2.4-mm Locking Compression Plate Distal Radius System, ® dorsoradial and dorsoulnar).

In the present series, flexion was slightly limited. Our results are compatible with previously reported studies comparing volar and dorsal

plating [3, 5]. This finding may be the result of dorsal capsular contracture and/or adhesion of extensor tendons.

Our results revealed that the treatment of displaced intra-articular distal radius fractures with a dorsally placed interlocking plate system demonstrated similar objective and subjective clinical results, except for the loss of wrist flexion. Furthermore, the overall complication rate was not high in the dorsally placed interlocking plate. Certain fracture patterns are more appropriately stabilized with dorsal plate fixation, but the indication is less frequently applicable than that for volar plate fixation.

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Chapter 5

COMPLEX PHYSIOTHERAPEUTIC AND REHABILITATION PROGRAMME INCLUDING LABOR ACTIVITIES IN PATIENTS AFTER DISTAL RADIUS FRACTURE

Danelina Emilova Vacheva, PhD
Department for Physical Medicine, Rehabilitation,
Ergotherapy and Sports, Medical University
Clinic for Physical Medicine and Rehabilitation
University Hospital, Pleven, Bulgaria

ABSTRACT

Introduction: The interest in rehabilitation of patients recovering after distal radius fracture is determined by the importance of the upper limp for performing all daily activities. The achievements of contemporary physiotherapy and rehabilitation medicine are significant and the scientific research in this direction is realized in different aspects. The main focus is on investigating combinations of methods and resources for a quicker and complete functional recovery.

Material and methods: Our research included 106 patients divided into two groups (with and without labor activities) – 51 patients had a traumatized dominant hand and 47 had a complication of M. Zudeck.

For over sixty years the Clinic of Physical and Rehabilitation Medicine at the University Hospital in Pleven (Bulgaria) has been applying a complex physiotherapy and rehabilitation program. It includes: *Sub water gymnastics* – local bath tub with water temperature 34-36°C (in the area of wrist joint and forearm); *Kinesitherapy*; *Labor activities* (occupational therapy); *Impulse magnetic field* (15-20 min, 2 A, 1-100 Hz); *Interference currents* (5 min, 90-100 Hz; 10 min, 1-100 Hz); conducted daily for 10 days.

The results are analyzed immediately after removal of the gypsum immobilization and at the end of the rehabilitation process. We have designed a special test to research and report *Comprehensive Functional Status* of patiens after trauma and diseases of the upper limb, including contractions in radioulnar joints, wrist joint, finger and thumb joints. The test contains five sections: (1) assessment of pain; (2) volume of movement of radioulnar joints, wrist joint, fingers and thumb; (3) MMT (Manual Muscle Testing) of pronation and supination in the radioulnar joints, flexion and extension in wrist joint and the muscles of the fingers and thumb; (4) tests of the grips; (5) Daily Life Activities (DLA) with separate parts for toilet and personal hygiene, dressing and shoes, preparing food and meals, various household activities. The maximum number of 100 points is an "Excellent" functional recovery.

Results: The results obtained before and after rehabilitation, with damage to dominant and non-dominant upper limbs (with or without complication of M. Zudeck) show "Very good" and "Excellent" functional recovery of patients rehabilitated with inclusion of occupational activities, and "Good" recovery of those rehabilitated without it. In the case of complications of M. Zudek, neuritis of the n. median and shoulder-arm syndrome, the patients were treated with a complex rehabilitation program including labor activities up to 0,7 months (21 days), and in non-occupational patients – 1,1 month.

The analysis of the results obtained gives us grounds to confirm, with high statistical significance, the medical and social effectiveness of the proposed complex Physiotherapeutic Rehabilitation Program including occupational activities in the treatment of patients with distal radius fracture.

Conclusion: Improving the patient's quality of life requires the complete functional recovery of the arm, which leads to the restoration of the patient's overall personality, his psyche, working capacity, social and economic status.

Keywords: fracture, distal radius, rehabilitation, ergotherapy, occupational therapy

INTRODUCTION

The interest in rehabilitation of patients recovering after distal radius fracture is determined by the importance of the upper limp for performing all daily activities. The achievements of contemporary physiotherapy and rehabilitation medicine are significant and the scientific research in this direction is realized in different aspects. The main focus is on investigating combinations of methods and resources for a quicker and complete functional recovery [27].

MATERIAL AND METHODS

Our research included 106 patients devided into two groups (with and withour labor activities) - – 51 patients had a traumatized dominant hand and 47 had a complication of M. Zudeck.

For over sixty years the Clinic of Physical and Rehabilitation Medicine at the University Hospital in Pleven (Bulgaria) has been applying a complex physiotherapy and rehabilitation program, which includes:

- Underwater gymnastics – local bathtub with water temperature 34-36°C (93-97°F) in the area of wrist joint and forearm;
- Kinesitherapy;
- Labor activities (occupational therapy);
- Impulse magnetic field (15-20 min, 2 A, 1-100 Hz);
- Interference currents (5 min, 90-100 Hz; 10 min, 1-100 Hz); conducted daily for 10 days.

1. METHODOLOGY OF UNDERWATER GYMNASTICS

For conducting underwater gymnastics we use a *local bathtub;* the patient is in starting position – side-sitting, injured side close to the bathtub, extremity immersed in water, if possible up to the middle part of the armpit (Figure 1). Water buoyancy alleviates the action of muscles, weak and hypotonic due to immobilization, and hydrostatic pressure softens the periarticular tissues and helps handling contractures [7]. Water temperature is average (indifferent) – 34-36^{0}C (93-97°F) [2, 31]. The exercises are analytic for all muscle groups in forearm, fingers and thumb, and we fix the proximal segment of the extremity to support a precise and proper performance of the exercises. In the beginning, the light exercises are repeated 5-6 times, at a very slow rate, and performed without pain and excess tension. As recuperation progresses, number of repetitions, series of exercises and rate of performance are increased, the duration of procedure is prolonged as well. From 10-12 minutes at the beginning of the rehabilitation, to 20 minutes, depending on patient's individual status and period of recuperation he is in – early or late post-immobilization period [23].

Figure 1. Conducting underwater gymnastics in a local bathtub.

2. METHODOLOGY OF KINESIOTHERAPY

The purpose of kinesiotherapy, carried out on patients after fracture of distal radius is to recuperate the arm to the fullest possible extent so that the patient can return to everyday life and work process (Figure 2). The kinesiotherapy methodology is consistent with damage level of the whole upper extremity, any complications or concomitant diseases, period of recuperation, age, occupation, individual abilities, intellect and adaptation abilities of the patient [21].

Figure 2. Conducting kinesiotherapy.

2.1. Early Post-Immobilization Period

The immediate period after the immobilization unit is removed is considered as early post-immobilization period and continues for two or three weeks – this is the time of the *first rehabilitation course*. The tasks we set are: affecting the pain syndrome; improving movement habits and skills for hygiene and self-service; boosting the psychological and emotional state of the patients; beating the forearm swelling and improving tissue trophic; recovering the range of motion of affected joints – radioulnar, wrist joints, fingers; getting over the contractures through adequate mobilization of the

radioulnar joint, the wrist joint and the fingers joints; improving functionality of various grips, mostly the grasping ones; beating the muscle imbalance; cutting the option for development of algoneurodystrophy complications (M. Zudeck and shoulder-arm syndrome) [4].

The main *tools* of the kinesiotherapy in this period of patient rehabilitation are mainly the *active physical exercises*, toning the whole upper extremity, performed at first in suspension, in effluent starting position, without training equipment, and later with suitable gym equipment and means. The special exercises are alternated with breathing and relaxing exercises, and at the same time the principle *from proximal to distal joints* shall be followed in order to affect the swelling [14]. Passive, redressing movements causing pain are not performed as such behavior is methodologically wrong for patients with fracture of the distal radius. Pain, as well as other abrupt irritations and interventions in the damaged area (hot procedures, rough or energetic massage) very often can trigger the Zudeck algoneurodystrophy syndrome. Such type of fractures in early post-immobilization period need mainly a dosed active kinesiotherapy and in particular suitable domestic and work activities, without excessive tension.

In the beginning of the rehabilitation course the exercises are fewer in number, and performed at a slow to moderate rate, with fewer repetitions and longer pauses, from position relieving the relevant muscles and muscle groups, within unpainful range of motion for the separate joints, without substituting or compensating movements, clear and precise performance.

The kinesiotherapeutic set of exercises starts from 10-15 minutes in the beginning of the therapy, and extends to 20-25 minutes in the end of the rehabilitation course. The patients are given instructions on how to facilitate their everyday tasks in domestic environment.

2.2. Late Post-Immobilization Period

During the late post-immobilization period (main) that comprises the time frame for the *second rehabilitation course*, usually at the end of the second and the beginning of the third month after the trauma

(immobilization period included), the following ***tasks*** are set: getting over the remaining contractures in joints; maximum restoration of trophic of the damaged arm; strengthening weak and hypotrophy muscles and muscle groups and from there the power of the grips; improving coordination of fingers' fine movements (precise grips) and engaging the arm in everyday domestic and work activities [8, 22].

We expand *the range of means* used in this period. Apart from active gymnastic exercises with gym equipment, we include manual mobilization of all peripheral finger joints and of the wrist joint, counter-resistance exercises, techniques from the methodology of post-isometric relaxation, proprioceptive neuro-muscle facilitation and mechanotherapy.

Dosage of load is increased as well. Duration of the procedure in kinesiotherapy is 30-40 minutes, the exercises are performed at a moderate rate, with less and shorter pauses, more repetitions, up to 2-3 series. To strengthen certain muscles and muscle groups we use counter-resistance exercises assisted by the kinesiotherapist or with the use of small weights (0,5-1 kg) and isometric contractions.

2.3. Kinesiotherapy in Case of M. Zudeck Complications and Shoulder-Arm Syndrome

The *tasks* we set for patients who have developed the M. Zudeck algoneurodystrophy syndrome are: affecting the pain, improving blood and lymph circulation and alleviating the edema; stimulating capillary blood circulation; stimulating the tissue trophic and faster formation of bone substance; affecting through reflector methods the neurovegetative balance; preventing contractures aggravation; stimulating the participation of the damaged extremity in everyday activities, without overloading, which leads to improved psychological and emotional condition of the patient [1].

The means used for this kinesiotherapy treatment set are more specific; here we include treatment through positioning, exercises in suspension for the whole upper extremity, exercises from effluent starting position, isometric contractions for larger muscle groups of the upper extremity,

analytic exercises at eliminated gravity, fly exercises and relaxation techniques.

The dosage of the kinesiotherapy procedure for patients with complications is estimated very carefully. Overloading is unacceptable. The exercises are elementary in coordination aspect, in the proper movement planes, without pain and redressing, performed proactive only, or facilitated and assisted. Small number of repetitions, frequent pauses, for e longer period, slow to moderate rate of performance, depending on the rate of recuperation. The number of procedures is not to be overdosed, as it would have adverse effect.

For patients who have developed the shoulder-arm syndrome we apply more special kinesiotherapy program that targets mainly contractures in shoulder joint [6].

3. Methodology of Electrical Stimulation Procedures

Electrical stimulation treatment improves blood supply, stimulates tissue trophic and metabolism, stimulates nerve receptors and transmission of impulses via nerve-muscle synapse, it has painkiller effect, stimulates organ and system functions.

3.1. Application Method of Low-Frequency Pulsating Magnetic Field (LFPMF)

Physiological effect of LFPMF (depending on dosage) is expressed in suppression of increased nervous excitation causing analgesia; relaxation of muscle spasm; improved trophic, metabolism, tissue regeneration, and has anti-inflammatory effect (Figure 3).

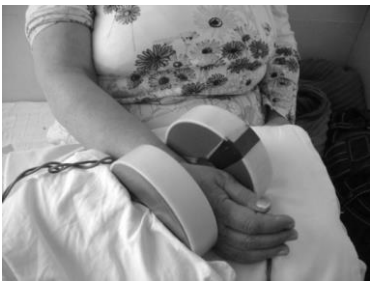

Figure 3. Conducting of Low-Frequency Pulsating Magnetic Field.

Treatment is performed through two inductors placed on wrist joint sides, hand is placed on a small cushion, for 20 minutes, at administered dosage depending on apparatus type (apparatus Magnit 80 – III degree (204,66 Oe, 1,2A, period – 1 sec, pulse: period – 2:10; scope – x 1 – 10 sec) 20 minutes or apparatus Magnit 87 – 20 min 2A 1-100 Hz. When applying magneto therapy in fracture cases, we rely on osteogenesis stimulation [28].

3.2. Application Method of Interferential Current Therapy (ICT)

The method for ICT that we employ includes four electrodes, 50 sq. cm area, allocated in a way that the fracture area is situated in interference zone of the two medium frequency currents (Figure 4). The current frequency is 100 Hz for 5 minutes, and its intensity is according to patient's individual sensitivity, he shall feel a nice, soft massage. Then the frequency is switched to 90-100 Hz for 10 minutes.

The healing effect of the ICT is due to its anesthetic effect; the therapy normalizes the vascular tone and permanently improves the blood supply (including capillary area), increases the lymph flow, removes quickly metabolic toxins, helps for better oxygen supply to tissues, changes tissue pH to alkaline reaction, because it has been established that, especially in bone atrophy, acidosis may occur; it activates electrolyte metabolism (Ca, K, Na). Treatment with ICT has the advantage that it can be applied in cases of slow bone substance formation, as it stimulates osteogenesis on the basis of

vasodilatory effect which is very important condition for bone regeneration [15, 16, 19, 26].

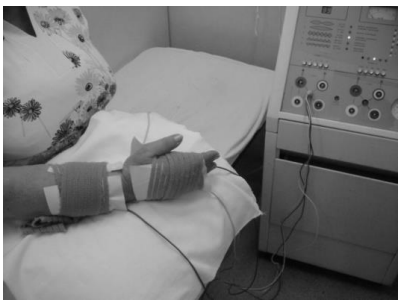

Figure 4. Conducting of Interferential Current Therapy.

In cases of severe form of M. Zudeck syndrome, the ICT helps for better oxygen supply to tissues, removes venous stasis, speeds up functional recuperation and normalized the bone structure.

The ICT removes very quickly the painful rigidity in joints after prolonged immobilization which makes it an excellent tool for preventing and getting over the contractures, inevitably concomitant to distal radius fracture.

4. Application Method of Occupational Therapy

Occupational therapy includes some types of work activities, depending on the period the patient is in, his individual functional abilities, personal preferences and the time he has for the activity [3, 9, 11, 17, 20]. It is conducted every day, after the other procedures from the rehabilitation set and shall continue 15-20 minutes in the beginning of the first course to 30-40 minutes and even more in the end of the recuperation process. The physical load of the damaged extremity is dosed according to patient's individual abilities; no rush and more breaks shall be given and the requirement to the quality at the beginning shall be lowered.

4.1. Facilitating Everyday Life Activities (ELA)

During the first days, in the beginning of the rehabilitation and immediately after removing the immobilization unit, our efforts are focused on providing instructions and training the patients how to use facilitating techniques for maintaining personal hygiene, completing basic tasks for self-service and other *everyday* domestic activities [5, 10, 29, 30].

The most fundamental and important thing for each patient with limited mobility of upper extremity is maintaining personal hygiene and self-service. The instructions we give to the patients in that aspect are to facilitate their everyday activities, to include the damaged arm, without sparing it, with moderate load.

- *Activities for maintaining personal hygiene:* using toilet, washing hands, washing face, teeth brushing, wiping hands (face) dry, combing hair, shaving, nails trimming, showering.
- *Activities for dressing and putting shoes:* dressing (undressing) underwear, dressing (undressing) blouse, putting outwear (jacket), putting skirt (trousers), clasping belt, buttoning (small and large buttons), putting (removing) socks, putting shoes on (off), tying (untying) shoes laces, putting gloves on, tying scarf up.
- *Activities related to cooking and having meals:* plugging on (off), switching a stove on (off), using water tap, washing utensils, pouring liquids in a cup, drinking from a cup, loading a dish with meal, using a spoon, using a fork, cutting food products with a knife, cleaning a table.
- *Various activities using the hands:* writing (with the active hand), opening (closing) the door, opening (closing) the window, turning the lock key on (off), going through a book (a newspaper), using matches, handling wallet and money, counting coins, using a handkerchief, switching a lamp on, handwashing, hanging up clothes to dry, ironing, using a telephone.

4.2. Methodology of Occupational Therapy and Work Activities

After a few days of rehabilitation, the patients start self-servicing with more confidence, feel improvement and gradually, other activities requiring more precise movements and strong grips are included in the therapy [12, 13, 18].

One of the easiest *work activity* is working with yarn (unraveling old knitted items) and working with paper. These activities are suitable for both men and women, of various ages, and engage both upper extremities equally, regardless which is the damaged extremity – the dominant or non-dominant.

In some patients recuperation goes faster and more successfully and we offer them to work with yarn and cotton fibres – crochet, knitting (Figure 5), embroidering on wooden frames (Figure 6), mostly to women, and to men – working with leather – manufacturing various objects through leather wrapping.

During second and next rehabilitation courses, along with everyday life activities, in work therapy procedures we offer working with plastic materials, working with wood or other activities as per their wish. Work therapy proved to be very effective especially when the patient, in the end of his treatment, has manufactured something - a product of his efforts that can actually be used in everyday life.

The results are analysed immediately after removal of the gypsum immobilization and at the end of the rehabilitation process. We offer specially designed by our test to research and reporting of *test for Comprehensive Functional Assessment of Radioulnar Joints, Wrist joints and Fingers*, Similar to the test UCLA (C. Rockwood, F. Matsen, 1998) [25] to functional assessment of the shoulder joint and test W. D. Regan (1991) [24] to functional assessment of the elbow and radioulnar joints, modified by N. Popov (2003) [21]. Comprehensive Functional Status, of patiens after trauma and diseases of the upper limb, including contractions in radioulnar joints, wrist joint, finger and thumb joints. Test contains five sections – (1.) assessment of pain; (2.) volume of movement of radioulnar joints, wrist joint, fingers and thumb; (3.) MMT (Manual Muscle Testing) of pronation

and supination in the radioulnar joints, flexion and extension in wrist joint and the muscles of the fingers and thumb; (4.) tests of the grips; (5.) Daily Life Activities (DLA) with separate parts for toilet and personal hygiene, dressing and shoes, preparing food and meals, various household activities.

The maximum number of 100 points is an "Excellent" functional recovery (Table 1).

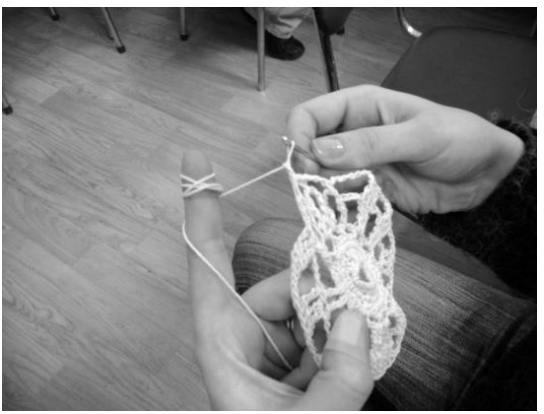

Figure 5. "Crochet."

Figure 6. "Embroidering."

Table 1. TEST for Comprehensive Functional Assessment of Radioulnar Joints, Wrist Joints and Fingers

№	Type of test	Test – points	Before	After	Difference
1.	Pain – 20 p.	0 p. – Strong / 20 p. – Lacking			
2.	Range of movement	Total – 20 p.			
	Radioulnar Joints	Supinatio – max. 3 p. (1° = 0,03 p.)			
	max. 6 p.	Pronatio – max. 3 p. (1° = 0,03 p.)			
	Wrist Joints	Extensio – max. 3 p. (1° = 0,04 p.)			
	max. 10 p.	Flexio – max. 3 p. (1° = 0,04 p.)			
		Rad. abd. – max. 2 p. (1° = 0,08 p.)			
		Uln. add. – max. 2 p. (1° = 0,04 p.)			
	CMC joint of the pollex	Extensio – max. 1 p.			
	max. 4 p.	Flexio – max. 1 p. (1° = 0,02 p.)			
		Abd. – max. 1 p. (1° = 0,01 p.)			
		Add. – max. 1 p. (1° = 0,06 p.)			
3.	Manual Muscle Testing	Total – 14 p.			
	Radio-ulnar Joint – max. 4 p.	Supinatio – max. 2 p. (1 gr. = 0,04 p.)			
		Pronatio – max. 2 p. (1gr. = 0,04 p.)			
	Wrist Joints – max. 6 p.	Extensio – max. 2 p. (1 gr. = 0,04 p.)			
		Flexio – max. 2 p. (1 gr. = 0,04 p.)			
		Rad. abd. – max. 1 p. (1 gr. = 0,02 p.)			
		Uln. add. – max. 1 p. (1 gr. = 0,02 p.)			
	CMC joint of the pollex – max. 4 p.	Extensio – max. 1 p. (1 gr. = 0,02 p.)			
		Flexio – max. 1 p. (1 gr. = 0,02 p.)			

Table 1. (Continued)

№	Type of test	Test – points	Before	After	Difference
3		Abd. – max. 1 p. (1 gr. = 0,02 p.)			
		Add. – max. 1 p. (1 gr. = 0,02 p.)			
4.	Tests grabs	Total – 19 p.			
	max. 2 p.	Spherical – (1 gr. = 0,04 p.)			
	max. 2 p.	Cylindrical – (1gr. =0,04p.)			
	max. 3 p.	Fist – (1 gr. = 0,06 p.)			
	max. 1 p.	As hook – (1 gr. = 0,02 p.)			
	max. 4 p.	Precision – (1 gr. = 0,08 p.)			
	max. 3 p.	Palmar – (1 gr. = 0,06 p.)			
	max. 3 p.	As key – (1 gr. = 0,06 p.)			
	max. 1 p.	Scissor – (1 gr. = 0,02 p.)			
5.	ADL	Totalo – 27 p.			
	max. 6 p.	Toilet and personal hygiene – (1act. = 0,7 p.)			
	max. 6 p.	Putting on shoes and clothes – (1act. = 0,5 p.)			
	max. 7 p.	Preparing food and feeding – (1act. = 0,6 p.)			
	max. 8 p.	Daily and labour activities – (1 act. = 0,6 p.)			
	Total–max100 p.				
	Index	85 – 100 p. – Excellent, 70 – 85 p. – Very good, 40 – 70 p. – Cood, Less than 40 p. – Satisfactory			

RESULTS

Table 2 displays rate of improvement in patients after completed treatment (F = 9,65, P = 0,0001). The highest gap in the results is for patients who suffer complications with damaged dominant extremities, which can be explained by their much aggravated state immediately after immobilization is removed (beginning of rehabilitation).

Table 2. Differences from the end and the beginning of the rehabilitation process from the Comprehensive Functional Assessment of Radioulnar Joints, Wrist Joints and Fingers

	n	X	S	Coeff var. %	P
Without complication dominant extremities	14	24,4	5,11	20,98	= 0,0001
Without complication non-dominant extremities	20	21,2	3,94	18,57	= 0,0001
With a complication dominant extremities	17	27,4	3,95	14,44	= 0,0001
With a complication non-dominant extremities	12	28,7	4,33	15,11	= 0,0001

Figure 7 displays the results from Complete Functional Assessment in the end of the rehabilitation process. The results show that patients without complications have the best functional state, assessed as “Very good.” 10% assess their rehabilitation state as “Excellent,” regardless which extremity has been damaged. Only 5% of patients with damaged dominant extremity assess their recuperation as “Good.” This proves that patients with dominant extremities in the end of the treatment show better recuperation in comparison to non-dominant ones (P < 0,05). Lowest results are shown in patients with damaged non-dominant extremities and with complications. All patients remain in “Good” functional state. 30% of these with dominant extremities show better results and assess their recuperation as “Very good.”

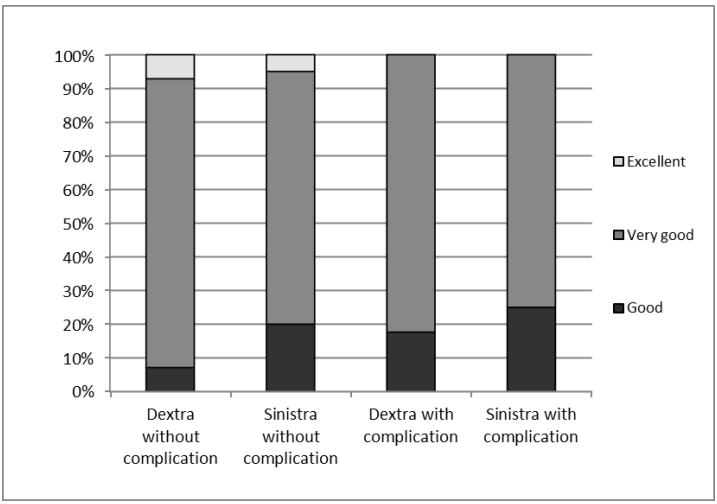

Figure 7. Results from Complete Functional Assessment at the end of the rehabilitation process.

The analysis of the results obtained gives us grounds to confirm, with high statistical significance, the medical and social effectiveness of the proposed complex Physiotherapeutic Rehabilitation program with inclusion of occupational activities in the treatment of patients with distal radius fracture.

CONCLUSION

Improving the patient's quality of life requires the most complete functional recovery of the arm, which leads to the restoration of the patient's overall personality, psyche, working capacity, social and economic status.

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Chapter 6

EVALUATION OF SYMPTOMATIC AND ASYMPTOMATIC MECKEL'S DIVERTICULUM

Anthony J. Ferrara[1], MD and
Sathyaprasad Burjonrappa[2,*], MD
[1]Department of Surgery, Stony Brook University,
Stony Brook, NY, US
[2]Department of Surgery, The Children's Hospital at Montefiore,
Einstein College of Medicine, Bronx, NY, US

ABSTRACT

Meckel's diverticulum (MD) is the most common congenital anomaly of the gastrointestinal tract, affecting approximately 2-4% of the population. It is the result of an incompletely obliterated vitelline or omphalomesenteric duct around weeks 5-8 of gestation. Approximately 60% of Meckel's diverticula contain heterotopic mucosa, most commonly acid-secreting gastric mucosa. The lifetime incidence rate of complications from a Meckel's has been cited to be 4-6%. Common clinical presentations constituting symptomatic Meckel's are intussusception, volvulus, internal hernia, adhesions, Littre hernia, gastrointestinal bleeding (the most

* Corresponding Author E-mail: sathyabc@yahoo.com.

common presentations leading to the discovery of a Meckel's), diverticulitis, and perforation. Most Meckel's diverticula are asymptomatic and are discovered incidentally during imaging, endoscopy, or at the time of a surgical procedure. Technetium-99m pertechnetate scintigraphy (Meckel's Scan) has been utilized to attempt localization of the diverticulum. However reported sensitivities are as low as 60% but specificity is as high as 90-98%. Capsule endoscopy (CE) has come in vogue recently but is beset with deterring complications including risk of delayed passage or obstruction requiring surgical retrieval of the device. Other commonly used diagnostic modalities include plain abdominal radiographs, abdominal ultrasound, CT and MRI. Magnetic resonance enterography (MRE) has risen in popularity, particularly in bleeding patients, with a sensitivity in the range of 70% for localizing the site of origin of bleeding. For symptomatic patients, many advocate going to the operating room for exploration. Common indications for diagnostic laparoscopy or laparotomy are recurrent GI bleeds, chronic abdominal pain or persistent leukocytosis of unclear etiology, idiopathic ileus or persistent bowel obstructions, and repeated intussusceptions. In the case of bleeding, tagged RBC scintigraphy or angiography could also be utilized to determine the source of bleeding and possibly direct the clinician towards Meckel's. In brief, diagnosing Meckel's diverticulum can be very challenging, especially as the majority are asymptomatic, and those that are symptomatic can masquerade as other clinical entities such as appendicitis or bowel obstruction. Radiologic and nuclear medicine studies can be of use, however if clinical suspicion is great enough, diagnostic laparoscopy will likely yield the diagnosis in a more direct fashion.

Meckel's diverticulum (MD) is the most common congenital anomaly of the gastrointestinal tract, affecting approximately 2-4% of the population. Of those with symptomatic MD, half will develop symptoms before age two years and most complications develop before ten years of age. In our experience, ectopic epithelium is not present in MD that were excised in the neonatal period and early infancy, but present in the majority of MD excised after one year of age [26]. Most MD are asymptomatic however, and are incidentally discovered on imaging, during endoscopy or at the time of a surgical procedure. The most common presentation of symptomatic MD is bleeding per rectum. Indeed, nearly 50% of all lower GI bleeds in children are caused by MD, most commonly in infants and toddlers. The color of the stools is inconsistent with rectal bleeding and can be bright red, maroon/dark

or even tarry [15]. Other potential manifestations of symptomatic MD can be intussusception, volvulus, internal hernia, adhesions, Littre hernia (inguinal hernia sac contains the MD), diverticulitis, and perforation [3].

Charles Mayo famously wrote in 1933 that "Meckel's diverticulum is frequently suspected, often looked for, and seldom found [16]". Most clinicians will keep MD high in their differentials when faced with a child with lower gastrointestinal bleeding. Like any patient, one first begins with a thorough history and physical examination. One should elicit timeline of symptom onset, rule out any history of trauma or abuse and determine if the patient had any complications during the pregnancy or after which may predispose to bleeding. Physical examination tends to be non-specific, and in the case of Meckel diverticulitis, exam is remarkably similar to that of acute appendicitis, obviously confounding the clinical picture. Therefore, the next step is imaging.

Plain radiographs are unlikely to demonstrate more than signs of bowel obstruction or the occasional enterolith. Small bowel follow through (SBFT) with barium gave greater detail but have been supplanted by newer and more reliable techniques. With barium, the MD would appear as a blind-ending pouch originating from the anti-mesenteric border of the distal ileum. Filling defects could sometimes be noted within the diverticulum and would be suggestive of ectopic gastric mucosa or neoplasm [17]. In similar fashion, Sato and colleagues have recently described diagnosing MD utilizing endoscopic retrograde ileography with gastrografin [18]. The colonoscope tip was inserted into the terminal ileum and 150 cc of dilute gastrografin was instilled and the MD was visible within 10 minutes fluoroscopically.

In children, oftentimes plain ultrasound is the first line modality in an attempt to limit or eliminate the need for radiation. Particularly, as Meckel diverticulitis closely mimics acute appendicitis in clinical presentation, many children with Meckel diverticulitis will receive an ultrasound examination. Though not very sensitive, MD will appear as a blind-ending tubular structure with hyperechoic mucosa and submucosa, and hypoechoic muscular layer (the so-called bowel signature) [19].

Most MD are discovered incidentally on CT. CT has a low sensitivity for detecting uncomplicated or normal MD [20]. With the advent of

multidetector and dual energy CT technology, the high resolution images enabled radiologists to examine finer slices of bowel from multiple perspectives, dramatically increasing the sensitivity of the modality. Additionally, dual energy technology has enhanced the ability of IV contrast to depict hemorrhage from MD, and with increasingly lower doses of contrast being necessary to do so. CT enterography may improve detection of MD because it combines the advantages explained above with the sensitivity of the previously popular barium SBFT to dramatically improve sensitivity [21, 22]. When seen on CT, MD appears as a blind-ending triangular or tubular outpouching from the distal ileum arising from the anti-mesenteric wall.

Pertechnetate scintigraphy (Technetium-99m), or Meckel scan, is the gold standard diagnostic tool for bleeding MD at this time. The radionuclide is administered intravenously and is taken up by the mucoid cells of the ectopic gastric mucosa and is subsequently excreted into the bowel lumen. This causes the ectopic gastric mucosa to enhance on imaging leading to diagnosis of MD. Normal uptake is obviously seen in the normal gastric mucosa and the proximal small bowel to a lesser extent. Abnormal uptake can also be seen with duplication cysts containing ectopic gastric mucosa, angiodysplasia, intussusception, or inflammatory bowel disease. Notably and importantly, uptake in bleeding MD is more focal and intense than in either of these previous scenarios. In one series, specificity and sensitivity of Meckel scan was 100% [23]. More typically, sensitivity is reported to be 85-90% in children versus around 60% in adults [22]. Sensitivity can be increased using several pharmaceutical agents. H2 receptor antagonists inhibit release of pertechnetate into the bowel thereby concentrating it in the tissue. Pentagastrin enhances localization in the bowel. Of note, H2 blockers and pentagastrin should not be given together or they will antagonize each other. Glucagon suppresses wash away of the pertechnetate by reducing bowel peristalsis. A Meckel scan should not be obtained in the setting of brisk bleeding as the radiotracer can be washed away rendering the scan non-diagnostic.

Mention should be made of capsule endoscopy (CE), as it has been popularized in the last few years but is not without its complications. Wireless capsule endoscopy involves swallowing a pill camera and allowing it to traverse the entirety of the GI tract after which the capsule is recovered in the stool. Sensitivity varies greatly by case series but in one series CE detected source of G bleeding in 44.6% of patients, and detected MD in 18.6% of patients [24]. A common complication of CE is retention of capsule that might result in bowel obstruction or perforation [25]. For this reason, CE is not as popular as it once was, especially given the low sensitivity of the study.

There are instances where imaging is negative and there is clear evidence that a patient is bleeding e.g., downtrending hematocrit, persistent tachycardia or hypotension despite infusion of crystalloid and blood products, gross passage of blood per rectum. In this case endoscopy is routinely performed in an effort to localize bleeding and obtain hemostasis. Interventional radiology can also attempt angiography and embolization however this can come with a substantial dye load which may be detrimental to the patient. Diagnostic laparoscopy or laparotomy can be performed in an attempt to identify a source of bleeding and resolve it. Not infrequently an extraluminal pathology can be identified such as an ulcerated diverticulum, AVM, or occult neoplasm.

In summary, two percent of MD are symptomatic and more than half of symptomatic MD contain ectopic gastric mucosa and bleed. Asymptomatic MD typically go unnoticed unless incidentally discovered when working the patient up for something else and are typically left alone in that case. If a child is found to have lower GI bleeding, MD is always suspected and a Meckel scan is obtained which will typically make the diagnosis. Other imaging modalities which have the potential to diagnose MD, albeit less sensitively, are ultrasound, CT or MRI with or without enterography, and CE.

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Chapter 7

SMALL FISH, BIG IMPACT: INSIGHTS FROM ZEBRAFISH ON HUMAN DISEASES AND DRUG DISCOVERY

Parth Thakkar[1], Chloe Shay[2,3] and Yong Teng[4,5,6,*]
[1]Department of Biology, College of Science and Mathematics,
Augusta University, Augusta, GA, US
[2]The Robinson College of Business,
Georgia State University, Atlanta, GA, US
[3]Emory Children's Center, Emory University, Atlanta, GA, US
[4]Department of Oral Biology, Dental College of Georgia,
Augusta University, Augusta, GA, US
[5]Georgia Cancer Center,
Department of Biochemistry and Molecular Biology,
Medical College of Georgia, Augusta University, Augusta, GA, US
[6]Department of Medical Laboratory, Imaging and Radiologic Sciences,
College of Allied Health, Augusta University, Augusta, GA, US

[*] Corresponding Author E-mail: yteng@augusta.edu Tel: +17064465611, Fax: +17067219415

ABSTRACT

The development and process of diseases in zebrafish (*Danio rerio*) is strikingly similar to those of humans, allowing human diseases to be easily modeled in zebrafish by induction of chemical mutagens, transplantation or by genetic manipulations. Therefore, zebrafish have been gaining popularity as one of the favored animal model systems for studying human disorders and developing novel strategies against them. This chapter outlines advances that have been made within the growing field of zebrafish as a model for human diseases and highlights their advantages for drug discovery.

Keywords: zebrafish, animal model, human diseases, drug discovery

1. INTRODUCTION

Given the physical and ethical problems with performing experiments on human patients, biomedical research relies on the use of animal models to study the pathogenesis of human diseases and to develop and test effective new therapies. To date, biomedical research has focused on using model organisms, with common ones being the mouse and the rat. These animals have been used due to the homology within mammalian genomes and similarities spanning in aspects from anatomy to cell biology to physiology. While these model organisms offer many advantages to modelling human diseases, they are expensive to maintain, difficult to manipulate, and limited for large scale genetic studies. As such, research has begun to look towards invertebrate model organisms to offer a way to overcome some of these disadvantages. There is a degree of functional conservation in basic cellular and biological processes between mammals and invertebrates, which suggests that diseases that result from the disruption of conserved cellular processes can be accurately modelled at a genetic and molecular level. Due to this, large scale forward genetic studies can be successfully done to determine gene functions and provide insight into how orthologous human disease genes function. Despite this huge advantage, invertebrates lack many structures and organ systems seen in mammals, and so their role in model-

ling human diseases is limited. While forward genetic mutation studies can be done in mammalian model organisms, the high cost and infrastructure support needed to do so is not commonly available. As such, zebrafish have come to attention as a model organism that can surpass most of these disadvantages mentioned, while retaining most of the advantages (Xie et al., 2012; Xie et al., 2015).

Besides the advantages of small size, low cost, and easy maintenance, the zebrafish has multiple additional benefits. Zebrafish share a high level of genetic and physiologic homology with humans, including brain, digestive tract, musculature, vasculature, and an immune system (Santoriello & Zon, 2012; Xie et al., 2015). Moreover, approximately 82% of all human disease genes have functional homologs with the species (Teng et al., 2013). Zebrafish are also fertile reproducers with the potential to produce over 100 embryos per clutch. Their development outside the uterus is rapid, with the major organs of the zebrafish becoming fully developed by 24 hours post fertilization (hpf), and they are ready for use in larvae experiments by 3 days post fertilization (dpf). Additionally, the small size of eggs and larvae allow easy fitting into standard 96-well microtiter plates, making zebrafish possible to use in large-scale small molecule screens (Fidler, 2003; Xie et al., 2015). With these advantages, it is easy to maintain thousands of zebrafish larvae in a laboratory at a reasonable cost for studying human disorders and revealing potential strategies to develop novel drugs to treat human diseases (Figure 1).

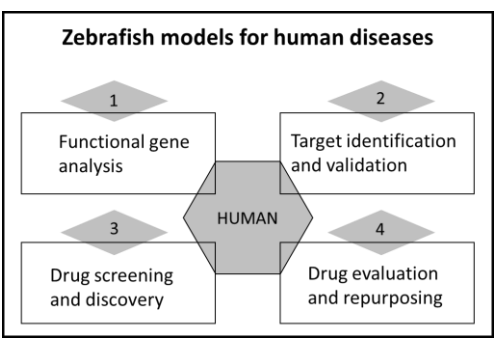

Figure 1. The promise of zebrafish as an alternative organism for modeling human diseases and drug development.

2. The Power of Zebrafish as Human Disease Models

2.1. Zebrafish as a Model of Human Cancer

Using mouse models for cancer research has led to several major drawbacks being noticeable, such as the effectiveness, the time requirements, and the expensive nature of mice. As such, zebrafish offer a viable alternative to mice as a model for cancer studies. It has been noted many genetic and pathological pathways associated with cancer progression in zebrafish share attributes with human cancer. Some tumor suppressor genes and oncogenes are conserved between zebrafish and humans, allowing zebrafish-cancer models to be a viable alternative to mouse models for studying human tumor development and progression (Shull et al., 2017; Teng et al., 2013).

An approach used to induce tumor formation in zebrafish involves dissolving or suspending chemical carcinogens into the water in which the fish are swimming in, and allowing the fish long term exposure to the water. The desired phenotype is either tumor formation or developmental defects. Studies have indicated zebrafish are the most responsive to carcinogens, showing a greater diversity of neoplasm types than other fish species. This method is advantageous as specific cancer types can be easily studied with minimal effort being required to induce them. Spitsberge et al., identified that explore of fry (3 week post-hatch) to 7,12-dimethylbenz[a]anthracene (DMBA) by immersion in aqueous solutions for 24 hours showed the widest diversity of target tissues and histologic types of neoplasia, having several types of epithelial, mesenchymal, and neural neoplasia. This study provides direct evidence that zebrafish act as a valuable model system for studying mechanistic aspects of the 3 carcinogenesis process (Spitsbergen et al., 2000). Larcher and colleagues found that chronic dietary exposure of zebrafish to polycyclic aromatic hydrocarbon (PAH) mixtures can induce carcinogenotoxic events that impair survival and physiology of exposed fish (Larcher et al., 2014; Teng et al., 2013).

As of now, only a few *in vivo* models are available where the individual stages associated with cancer progression can be followed in real time. Zebrafish is one of the few models that is able to do so. The adaptive immune system in young zebrafish does not fully develop until 14 dpf (Teng et al., 2013). The late development of the adaptive immune system and the ability to transplant human cancer cells in transparent zebrafish removes the hurdle of having to use immunosuppressed mice, which considerably reduces the time and cost of getting drug-tumor response data. This also allows human cancer cells to be xenotransplanted into fish embryos as early as 48 hpf and remain viable for up to 10 days. For better understanding cancer cell behavior in a living organism, the spread of fluorescently labelled human cancer cells through the zebrafish embryo can be clearly followed using microscopy.

Metastasis, which is the spread of cancer from the point of origin, is the primary cause of human mortality, accounting for approximately 90% of deaths (Fidler, 2003). There is evidence the metastasis phenotype is genetically controlled and independent of the process of cellular transformation. If specific targets can be found that inhibit this phenotype, targeting metastasis may be an effective method in reducing cancer mortality. Currently, most analysis of metastasis pathways occurs in controlled *in vitro* environments, through the overexpression or the removal of a specific gene (Teng et al., 2014). Assays have been developed that provide readouts on cellular phenotypes related to metastasis, but these phenotypes fail to address the issue of extravasation and intravasation processes requiring the use of an *in vivo* system. As such, any metastasis pathway identified in vitro must also be demonstrated as affecting invasion and metastasis *in vivo*. Recently, we established a metastasis model in zebrafish that recapitulated all steps of metastatic process observed in patients (Teng et al., 2013). Through the use of the zebrafish metastatic model, we evaluated the effect of combined expression of COP1 and GSK3βS9A in breast cancer metastasis (Shao et al., 2013). In this study, control of COP1/GSK3βS9A-expressing MDA-MB-231 cells were labeled with fluorescent dye CM-Dil, and approximately 200 labeled cells were microinjected into the perivitelline space of 48 hpf zebrafish embryos using

a pressure microinjector. By analyzing the extent of cancer cell dissemination in zebrafish, we demonstrated that simultaneously enhancing COP1 expression and elevating GSK3β activity can effectively suppress breast cancer metastasis (Shao et al., 2013). We also explored a critical role of short stature homeobox 2 (SHOX2) in breast cancer metastasis as the assessment of dissemination of SHOX2 knockdown and control breast cancer cells throughout zebrafish body (Hong et al., 2014).

2.2. Zebrafish as a Model for Epilepsy and CNS Disorders

Epilepsy is a common disorder which affects approximately 65 million people around the world (Banerjee et al., 2009). It can be characterized by mild seizures that last for a few seconds to strong seizures which can last up to several minutes, causing spasms, uncontrollable muscle twitches, and memory loss. Most current treatments revolve around treating epilepsy with antiepileptic drugs (AEDs). Although epilepsy can be successfully treated in most cases with AEDs, 30% of patients do not respond to available medical drug treatments. Therefore, there is an urgent need to identify drug mechanisms of AEDs and to develop more effective AEDs in order to improve treatment efficacy.

Zebrafish have a complex nervous system which is capable of complex behaviors and is susceptible to seizures. This allows zebrafish to be a prime model candidate for testing molecular mechanisms underlying cognitive deficiencies and potential therapeutic compounds. A study was done by Kundap et al., to use zebrafish as a model of epilepsy induced cognitive dysfunction and simulate clinical conditions which show both epilepsy and AEDs negatively affect cognitive function (Kundap et al., 2017). This model was developed to serve as a tool to develop and screen for newer, safer AEDs that do not have as many side effects. Epilepsy seizure-like condition can be induced in zebrafish using pentylenetetrazole (PTZ) (Teng et al., 2010; Teng et al., 2011). PTZ works by inhibiting the activity of gamma-aminobutyric acid (GABA) at GABAA receptors (GABAARs). In this study, the effects of different AEDs on locomotor activity and seizure-like activities were tested against zebrafish which had been induced seizures using PTZ.

Zebrafish were trained to associate light with shock stimulus in a fish shuttle box. T-maze tests were then used to study the learning skill, long and short-term memory, and memory plasticity in zebrafish (Roberts et al., 2013). The effects were determined using a T-maze test followed by neurotransmitter estimation and gene expression analysis. The experiment resulted in findings that overall, AEDs have a negative impact on cognitive functions, such as learning and memory.

Over the past decade alone, genetic predisposition to epilepsy has been linked to mutations in different genes. This complicated nature of epilepsy must be taken into account when developing therapeutic strategies. Most genetic predispositions to different types of epilepsy have been associated with mutations in different genes encoding structural components of ion channels. In 1995, Ottman and his colleagues described a rare form of epilepsy related to acoustic auras. This genetic disorder was described as autosomal dominant partial epilepsy with auditory features (ADPEAF) (Ottman et al., 1995). Leucine-rich glioma-inactivated protein 1 (LGI1) is a secreted protein containing a leucine rich repeat domain with cysteine clusters at the N-terminal and a beta propeller repeat in the C-terminal region (Teng et al., 2010; Teng et al., 2011). These motifs indicate a protein-protein interaction function, which makes LGI1 the first epilepsy susceptible gene identified that is not associated with ion channel proteins. Zebrafish models were used in a study done by our research group in 2010 to investigate how the knockdown of LGI1 results in seizure-like behavior and how embryonic development of the brain is affected. Zebrafish were chosen, as the LGI1 gene in zebrafish shows the exact high homology with, and the same exon structure as in humans, which suggests a conserved function of this gene. Interestingly, two paralogs of the LGI1 gene in zebrafish, lgi1a and lgi1b exist (Teng et al., 2010; Teng et al., 2011). Our study firstly focused on the lgi1a paralog, as it was the closest to the human gene in the phylogenetic tree analysis (Teng et al., 2010). The lgi1a knockdown zebrafish were generated using modified antisense oligonucleotides (MO). At lower doses of MO, the lgi1a morphants demonstrated the first two stages of seizures seen in zebrafish: rapid swimming activity and a circular ‘whirlpool’ swimming activity. At higher doses of MO, seizure-like phenotypes became

more pronounced. The phenotypic consequences of lgi1a knockdown resulted in abnormal development of the brain with increased apoptosis and abnormal eye development. These novel observations in zebrafish demonstrate that loss of lgi1a results in seizure-like activity, which may be due to its critical role in normal development of the brain.

With encouraging results coming from knockdown of the lgi1a gene, lgi1b zebrafish morphants were also generated by injection of specific MO into the one-cell embryos, which led to very different phenotypes (Teng et al., 2011). Although there were no plainly seen phenotypes present that pointed towards epilepsy, an increased hypersensitivity of lgi1b knockdown zebrafish morphants to PTZ was noted. Along with this, the lgi1b morphants showed delayed overall development, smaller eyes and brains, and increases apoptosis. The biggest notable difference in phenotypes was the increased size of the ventricles in the lgb1b morphants, leading to hydrocephalus. Loss of lgb1b in zebrafish during early development seems to cause these phenotypic changes to be permanent, as these changes were seen even when lgb1b expression levels recovered to normal. The different phenotypes between the two lgi1 morphants support a subfunctionalization model for the two paralogs.

2.3. Zebrafish as a Model for Cardiovascular and Metabolic Disease

Zebrafish models are also effective for cardiovascular research. Due to the embryos being nearly transparent, the internal structures can be observed without any invasive procedures. Moreover, the heart and blood vessels can be easily observed using microscopy, allowing quantification of cardiac contraction, blood flow, and vessel size. Two dimensional angiograms of most vessels can be generated without instruments using digital motion of the blood vessels. Compared with the technical difficulties found using this technique on vertebrates, the procedure is rapid and allows a good resolution of blood vessels. The resolution can be further amplified by using fluore-

scent dyes. The use of zebrafish instead of mice is practical and cost effective.

Like most animal models, zebrafish do not spontaneously develop cardiovascular diseases. However, isolated mechanisms thought to play a role in these diseases can be modeled and studied in an attempt to understand the processes of cardiovascular diseases. Thrombosis, the formation of a blood clot inside a blood vessel, is a mechanism that can be studied. Prothrombin knockdown by MO in zebrafish shows the same phenotype seen in knockout mice (Day et al., 2004), with spontaneous bleeding and a prolonged time for clot generation after injury. Moreover, researchers are able to induce localized thrombosis in zebrafish by targeted laser injury. Due to the clarity of the zebrafish embryo, real time formation of arterial or venous thrombus formation can be observed.

Arteriogenesis has been studied in mammalian models, but the results have not been satisfactory due to the difficulties of visualization *in vivo*. Ischemia has been debated as a driving force of arteriogenesis by researchers but could not be proven, because the arterial occlusion induced to stimulate arteriogenesis often results in downstream hypoxia (Deindl et al., 2001). Zebrafish embryo, which is able to oxygenate *via* diffusion, allows a satisfactory *in vivo* model for arteriogenesis to be created, as the embryo does not become hypoxic upon arterial occlusion. It is reported that inflammation plays a major role in cardiovascular diseases such as atherosclerosis and myocardial infarction (Frostegård, 2013). The interaction between inflammation and blood vessels can be easily studied in the zebrafish embryo, which does not have adaptive immunity until 4 to 6 weeks, and possesses innate immune mechanisms such as macrophage and neutrophil migration from 2 dpi. Transgenic zebrafish expressing the green fluorescent protein (GFP) in neutrophils have been used to study transmigration and neutrophil apoptosis at sites of inflammation (Shelef, Tauzin, & Huttenlocher, 2013).

3. THE POWER OF ZEBRAFISH AS AN *IN VIVO* DRUG DISCOVERY

Not only are zebrafish models good for investigating cancer pathways, they are also valuable assets in cancer drug discovery (Xie et al., 2015). Increasing studies have used the developing zebrafish embryo to determine the effects of therapeutic inhibitors during the developmental process of zebrafish. For example, an example to this approach comes from a study where a novel compound called lenaldekar (LDK) was used to inhibit the development of immature T-cells without disrupting the normal cell cycle progression of other cell types in the zebrafish (Ridges et al., 2012). Further studies following this experiment found LDK could be potentially used as a therapeutical method to treat certain types of leukemia. With the experimental directions zebrafish models can take, alongside their affordability and easy visual tracking of cellular process using fluorescent dyes, zebrafish offer several major advantages.

In the oncology field, finding the right treatment in the preclinical pipeline can greatly increase the quality of life and the probability of a patient surviving, as well as significantly reducing the cost of treatment. For example, the model was successfully used for chemical screening in clinical trials for the drug leflunomide, a compound that is used to treat melanoma. Recent efforts in cancer drug development have been directed at developing specifically targeting therapeutic drugs which not only are ideal in inhibiting specific disease driving mechanisms, but also demonstrate desired properties to be applicable in a clinical setting with patient applicability. ADP-ribosylation factor 1 (ARF1), one of small GTPases, is critical for breast and prostate cancer metastasis (Lang et al., 2017; Xie et al., 2016); therefore, inhibition of ARF1 function may have a potential therapeutic value in this regard. Due to the zebrafish-metastasis model and its capacity to evaluate the metastatic ability of cancer cells (Hong et al., 2014; Shao et al., 2013; Teng et al., 2013), we were able to assess the effects of a novel ARF1 inhibitor LM11 in zebrafish, which showed that 1 μM of LM11 significantly inhibited breast cancer MDA-MB-231 cells to disseminate from the perivitelline cavity to fish body (Xie et al., 2016). This study

showed, for the first time, that patients with breast cancer may benefit from ARF1 inactivation in the context of LM11 treatment.

Currently, druggable compounds used for targeted therapy can be divided into two fields: chemically synthesized small molecules and peptide-derived large molecule compounds. Small molecule compounds are those that can easily permeate the cell membrane and perform their derived function within the cell. Because of their low cost to produce and this advantage, small molecule compounds have found success in surpassing the hurdles needed to make a targeted inhibitor effective yet tolerable for patients. The Peterson's group developed a zebrafish model of doxorubin-induced cardiomyopathy recapitulating the cardiomyocyte apoptosis and contractility decline observed in patients, and conducted a counter-screen for cardioprotective compounds (Liu et al., 2014). Of the 3000 screened compounds, visnagin and diphenylurea were discovered from zebrafish assays, showing protected cardiac function without mitigating the chemo-therapeutic effects.

However, small molecule compounds are limited in their molecular specificity, often eliciting off target effects and are generally ineffective at disrupting protein-protein interactions (PPIs). Their ineffectiveness at disrupting crucial PPIs that promote oncogenic growth has led most small molecule compounds to be considered unusable in a clinical setting. To counter these drawbacks while still having all the advantages, peptide-based compounds are being investigated (Shull et al., 2017). These large molecule inhibitors are constructed to mimic molecules within the intracellular and intercellular environments. The compounds are designed to compete with the native protein domain interactions within the cell. Such peptide-based drug designs solve the issue of off-target effects that are caused by small molecule compounds, while still being able to disrupt molecular interactions with precision. The problem with large molecule compounds lies in their large size which makes them impermeable to cell membranes. Couple this with the high cost of designing and evaluating peptide-based therapies that are stable and maintain their shape *in vivo* due to stress of physiological conditions, the issues with this therapy can be noted.

Most recently, a great deal of effort is being put into making highly specific peptide-based therapies more stable and permeable, as many of the obstacles limiting the potential of these compounds is the physiological variability of *in vivo* environments. This, alongside the high cost of using mice and other vertebrates as *in vivo* models, has led to development coming to a halt. A proposed method to move past this obstacle is the use of zebrafish to determine the effectiveness or proposed therapeutic peptide compounds. Using the zebrafish xentransplanational model, *in vivo* drug studies assessing tumor growth and metastasis can be evaluated with relative ease and short timelines when compared to other models. This makes zebrafish a prime target for peptide-based compound trials. With the technical capabilities of both therapeutic peptide development and zebrafish-based cancer drug screening models advancing, in time these advances shall converge and allow zebrafish-based *in vivo* modeling to increase the potential of therapeutic peptide compounds in a clinical based setting.

FUTURE PROSPECTIVE

As noted, zebrafish possess a number of powerful advantages for biomedical research, and is particularly well-suited for studying human diseases with cellular resolution at the whole-animal level. With the ease of genetic editing or pharmacologic manipulations, human disease models in zebrafish would provide novel insights into the basic underlying pathological mechanisms. To help accelerate drug discovery and repurposing efforts for rare genetic diseases, zebrafish hold promise as a vertebrate model for primary drug screening at high throughput. In the future, zebrafish could may very well aid in target validation and drug discovery in this Era of Personalized Medicine.

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BIOGRAPHICAL SKETCH

Yong Teng, PhD

Affiliation: Augusta University, Augusta, GA, US

Research and Professional Experience: My areas of research include cancer metastasis, animal disease models and drug discovery. In my lab, we utilize zebrafish and mice to study molecular mechanisms of cancer metastasis, as well as drug actions and delivery, with the goal of

developing better therapeutics to treat human diseases, particularly in advanced cancer, epilepsy and spinal cord injury.

Professional Appointments: Assistant Professor, Department of Oral Biology, Dental College of Georgia, Augusta University Assistant Professor, Department of Biochemistry & Molecular Biology, Medical College of Georgia, Augusta University Assistant Professor, Department of Medical Laboratory, Imaging and Radiologic Sciences, College of Allied Health Sciences, Augusta University Member of Molecular Oncology and Biomarkers Program, Georgia Cancer Center

Honors:

2014 Prostate Cancer FY13 Idea Development Award, Department of Defense (DOD), US

2013 Basic Science Pilot Award, Georgia Cancer Center, US

Publications from the Last 3 Years:

*(out of 70 peer-reviewed articles, *corresponding author)*:

1. Lang L, Shull AY, Teng Y*. Interrupting the FGF19-FGFR4 axis to therapeutically disrupt cancer progression. *Curr. Cancer Drug Target* 2018 Mar 18. doi: 10.2174/1568009618666180319091731.
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Chapter 8

ZEBRAFISH MODEL USED IN FOOD SCIENCE AND TECHNOLOGY: ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES AND INHIBITION OF LIPID PEROXIDATION

R. Vilcacundo[2], D. Barrio[3], L. Piñuel[3], P. Boeri[3], D. Morales[1], I. Angós[2], A. Pinto[1], A. Castro[1], W. Reyes[1] and W. Carrillo[1,*]

[1]Research Department, Faculty of Health Sciences, Technical University of Babahoyo, Babahoyo, Ecuador
[2]Laboratory of Functional Foods, Faculty of Food Science and Engineering, Technical University of Ambato, Campus Huachi, Ambato, Ecuador
[3]Rio Negro National University, Viedma, Argentina

[*]Corresponding Author Email: wcarrillo@utb.edu.ec.

ABSTRACT

The zebrafish *in vivo* model presents many advantages when used in biotechnology and for food science and technology purposes. A large clutch size, transparent embryos, low-cost, fast growth and easy handling are the main features of this model. Recently, the zebrafish (*Danio rerio*) model, has been used to study vertebrate development and for modeling human diseases and processes such as oxidative stress, liver damage, inflammation and evaluation of inhibition of lipid peroxidation. Free radicals, such as superoxide (O_2), peroxyl (ROO), alkoxyl (RO), hydroxyl (HO), and nitric oxide (NO) play an important role in live organisms. Excessive free radicals induce various harmful effects in the human body such as cancer, liver injury, skin damage and aging. It is known that oxidative stress is caused by increasing the reactive oxygen species (ROS) which cause an imbalance with natural antioxidants that influences cell death and lipid peroxidation. Synthetic antioxidants, including butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have been used for food industrial purposes. However, recent research has focused on the extraction/identification of natural antioxidants from animal and vegetable sources. Recently, the zebrafish *in vivo* model has been used to evaluate the inhibition of lipid peroxidation using hydrogen peroxide and ethanol induced lipid peroxidation in quinoa, amaranth, red bean, milk proteins, walnut proteins, lysozyme and lysozyme peptides. For example, amaranth and quinoa have shown antioxidant activity and inhibition of lipid peroxidation in the zebrafish model. Proteins, hydrolysates and peptides from hen egg white lysozyme presented antioxidant activity and inhibition of lipid peroxidation in zebrafish larvae. Gastrointestinal digests from *Chenopodium quinoa* Willd and *Amaranthus caudatus* L. have reported lipid peroxidation inhibition in the zebrafish larvae model. Casein and whey protein hydrolysates from cow inhibited lipid peroxidation in the zebrafish larvae model. The zebrafish embryos model is an excellent model to evaluate the *in vivo* inhibition of the formation of ROS using a fluorescent method. Zebrafish embryos and larvae can be used to evaluate the cytotoxicity of the natural antioxidants. Zebrafish embryos and larvae have been used as an anti-inflammatory model using LPS as a pro-inflammatory inductor.

Keywords: zebrafish, quinoa, amaranth, inhibition of lipid peroxidation, anti-inflammatory activity

ZEBRAFISH DISEASE MODELS

Zebrafish is a small fish used in a home aquarium presenting a high resistance in captivity conditions. Zebrafish (*Danio rerio*) eggs have a fast development. Zebrafish embryos are transparent to be observed. At 2 h post fecundation (pfh), the first period of cell division (Figure 1A) can be observed. Chorion is the membrane protecting the embryo of external medium, allowing the interchange of molecules. After 12 pfd, brain, eyes, abdominal zone, spinal cord and the movement of the embryo (Figure 1B) can be observed. At 24 pfh, brain, eyes, heart and movement of heart, spinal cord, swim bladder, intestine and typical movement inside the chorion (Figure 1C) can be observed. At 48 pfh, the embryo, with all characteristic of zebrafish, can be observed. The typical pigmentation in the body can be observed, being possible to observe the circulation in the body (Figure 1D). Zebrafish embryos can be easily handled in multi-well plates. For the figures below, an osmotic embryo medium was used in all assays, E2 1X (15 mM NaCl, 0.5 mM KCl, 1.0 mM $CaCl_2 \cdot 2H_2O$, 50 µM Na_2HPO_4, 150 µM KH_2PO_4, 10 mM $MgSO_4$. $7H_2O$, 0.7 mM $NaHCO_3$ and 0.5 mg/L of methylene blue dissolved in distillated water) containing 1 mL of vehicle (0.1% DMSO) (Cunliffe, 2003).

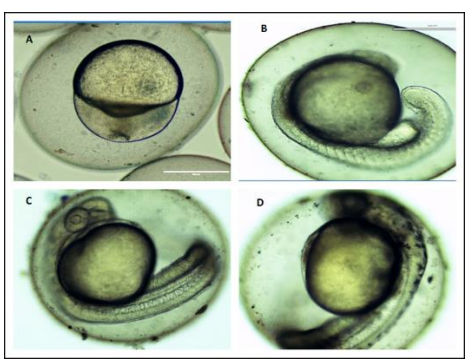

Figure 1. Photographs of eggs from zebrafish (*Danio rerio*). A) Zebrafish eggs after 2 post fecundation hours (2 pfh), B) Zebrafish eggs after 24 post fecundation hours (24 pfh), C) Zebrafish eggs after 36 post fecundation hours (36 pfh), D) Zebrafish eggs after 48 post fecundation hours (48 pfh).

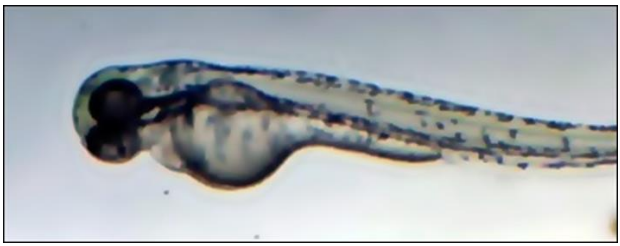

Figure 2. Zebrafish larvae 5 post fecundation days (pfd).

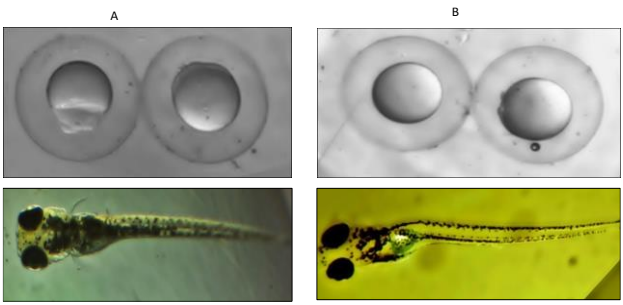

Figure 3. A) Zebrafish embryos and larvae without sample B) Zebrafish embryos and larvae incubated 96 h with sample.

Zebrafish larvae are used to evaluate cytotoxicity and biological activities, for their transparency and rapid development. Larvae are used when aged 5 post-fecundation days (pfd) (Figure 2). Figure 2 shows a typical zebrafish larva lateral photo. Figure 3 shows a zebrafish larvae (5 pdf) frontal photograph.

Zebrafish is widely used by pharmaceutical companies to determine the toxicity of new drugs and the toxicity of chemical compounds. The presence of heavy metals in waters can be evaluated with the exposure of zebrafish eggs and larvae. This animal model has had many uses. However, this model has been little used in food science. Recently, zebrafish (*Danio rerio*) has been used to evaluate the toxicity of isolated bioactive compounds from foods of plant and animal origins. Furthermore, this animal model has been used to evaluate certain biological activities of said bioactive compounds. Among the most studied biological activities are the antioxidant activity, inhibition of lipid peroxidation and anti-inflammatory activity.

TEST OF TOXICITY IN THE ZEBRAFISH MODEL

The wild type zebrafish colony was established in the laboratory of the Technical University of Ambato, in an environmental growth of glass aquarium, provided with a system of filtration and aerator activated carbon for water oxygenation. Adult zebrafish were kept to an exposition of 16 h light and 8 h dark cycles. Adult zebrafish were fed with commercial food and larvae of *Artemia saline*. Zebrafish embryos were obtained by photo-induced spawning over green plants and were incubated at 28 °C in a fish water tank. Early larvae post-fertilization zebrafish of 5 days were maintained at 28 °C and were observed with a microscopy.

Zebrafish of the AB strain (wild-type, wt) embryos were obtained from natural spawning. Embryos were raised and fish were maintained as described by Westerfield, (1995). After collection and disinfection, eggs were placed in 24-well microplates with 1 mL of water. To study the *in vivo* toxicity of all peptides coming from lysozyme with the zebrafish model, the Fish Embryo Toxicity (FET) test was employed.

FISH EMBRYO TOXICITY TEST

The assay followed the OECD guidelines chemicals on FET Test N° 236 of 2013 to zebrafish (*Danio rerio*). The test guideline was based on chemical exposure of newly fertilized zebrafish eggs for up to 96 hours and was expected to reflect acute toxicity in fish in general. 40 zebrafish embryos were used to evaluate five concentrations of the compounds. Different apical endpoints were recorded as indicators of acute lethality in fish: coagulation of fertilized eggs, lack of somite formation, lack of detachment of the tail-bud from the yolk sac and lack of heart-beat. The eggs are considered dead when they exhibit at least one of the previous mentioned indicators (Yang et al., 2018).

Yang et al., (2018) reported different extracts from *Polygonum multiflorum* Thunb with high toxicity against zebrafish embryos. This plant

is a traditional medicinal herbal used in East Asia for treatment of diseases associated with aging. This group assayed different ethanolic extracts and identified the components with high toxicity in the zebrafish model.

Carrillo et al., (2016a) described the evaluation of cytotoxicity of five peptides identified in hen egg white lysozyme (HEWL). These are five positively charged peptides f(109-119) VAWRNRCKGTD, f(111-119) WRNRCKGTD, f(122-129) AWIRGCRL, f(123-129) WIRGCRL and f(124-129) IRGCRL. All peptides presented cytotoxicity against zebrafish embryos. For example, the peptide with sequence f(122-129) AWIRGCRL produce 100% of mortality at a concentration of 5000 µg/mL. This peptide present no toxicity at a minimum assayed concentration of 50 µg/mL of peptide. The 50% of zebrafish embryos was affected at a concentration of 980 µg/mL after 24 hours of incubation (Figure 5). These sequences are rich in amino acid (Trp), (Cys) and (Arg). The sequence of the fragment f(124-129) IRGCRL has an absence of Trp. The antioxidant activity of these amino acids was evaluated using the ORAC method. Figures below show zebrafish embryos in presence of f(122-129) AWIRGCRL. Zebrafish embryos present coagulation stated with signal of cellular necrosis. The inhibition of lipid peroxidation of five peptides of HEWL was assayed in the zebrafish larvae model. All peptides were able of reducing the lipid peroxidation induced with hydrogen peroxide.

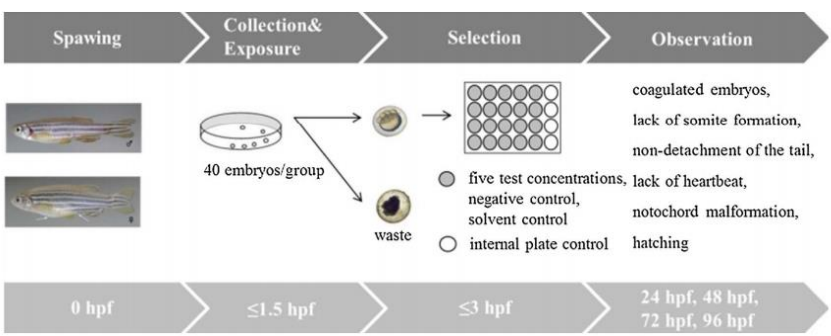

Figure 4. Acute toxicity in zebrafish embryos (Yang et al., 2018).

Vilcacundo et al., (2017) described the evaluation of cytotoxicity of hydrolysates from milk bovine proteins (native and denatured form) in

zebrafish embryos and larvae. Hydrolysates from casein milk and whey protein present an absence of toxicity in zebrafish embryos and larvae after 48 hours of incubation. The zebrafish embryos and larvae presented normal morphology. Malformations were not observed.

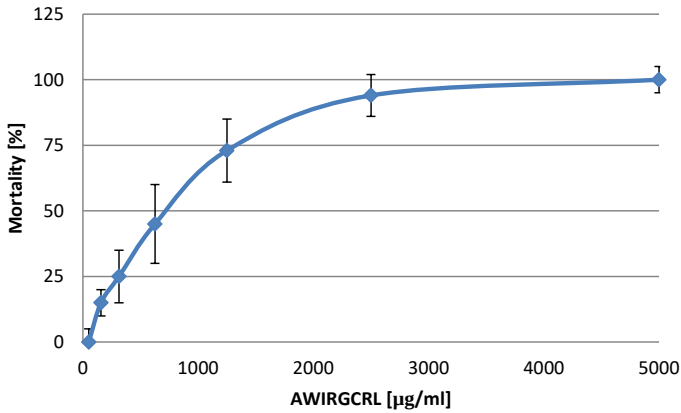

Figure 5. Acute of toxicity of zebrafish embryos incubated with synthetic peptide AWIRGCRL from HEWL for 48 h.

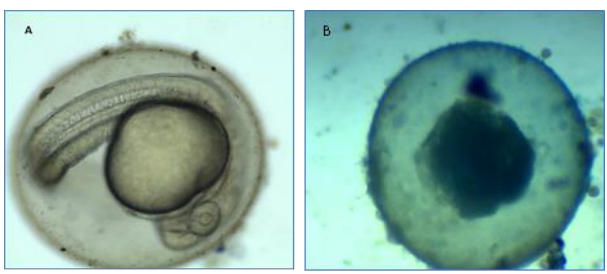

Figure 6. Morphological change observed in zebrafish embryos incubated with synthetic peptides AWIRGCRL from HEWL for 48 h.

Wang et al., (2016) described the evaluation of embryonic cytotoxicity of chitosan nanoparticles in zebrafish embryos (*Danio rerio*). The increased mortality and decreased hatching rate was found in the zebrafish embryo exposure to normal chitosan particles and chitosan nanoparticles with the increased addition concentration. At 120 h post-fertilization (hpf), the rate of mortality was 25.0% and 44.4% in the groups treated with chitosan

nanoparticles and normal chitosan particles at a concentration of (250 mg/L), respectively. At 72 hpf, the hatching rates in the groups treated with normal chitosan particles were lower ($P < 0.01$) at 300 and 400 mg/L than those of the corresponding control groups, respectively.

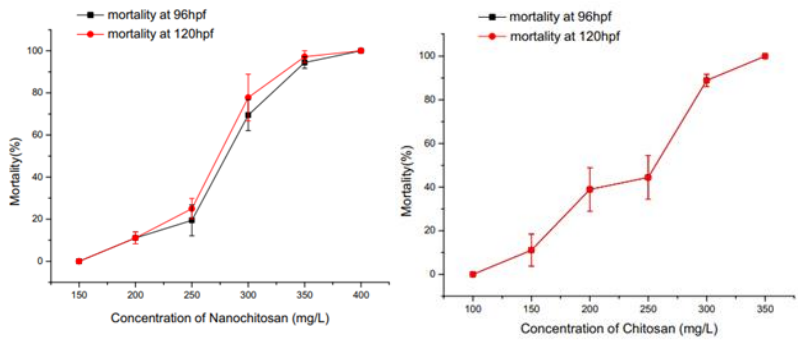

Figure 7. Acute of toxicity of zebrafish embryos incubated with normal chitosan and nanoparticle of chitosan (Yang et al., 2018).

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES AND INHIBITION OF LIPID PEROXIDATION

Lipopolysaccharide (LPS), has been used to induce inflammatory responses in different models such as cell lines and in the mice animal model. LPS has been used to establish an in vivo inflammation model in zebrafish for drug screening and bio-compounds. Yang et al., (2014) reported the use of the LPS agent to induce inflammation in zebrafish embryos. Park et al., (2011) have described the inflammatory effect of LPS in zebrafish embryos. High-density lipoprotein (HDL) and apolipoprotein (apo) A-I have strong antioxidant and anti-inflammatory properties in the plasma. HDL assayed in zebrafish embryos shows an increase in the survival rate of zebrafish embryos.

Reactive Oxygen Species (ROS) such as ($O2•^{-}$, HO2•, •OH named free radical) when they are found in large quantities, can produce oxidative stress conditions and be implicated in degenerative diseases such as Alzheimer and

Parkinson. This situation can induce a change in the presence of natural antioxidants with negative consequences such as damages in DNA and cellular lipid oxidation. Butylated hydroxy anisole (BHA), Butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ) and propyl gallate (PG) are antioxidant chemical substances widely used in the pharmaceutic and food industry to prevent lipid oxidation. In the last decade, their high cytotoxicity was evaluated in different *in vivo* models finding potential genotoxic effects. For this reason, their use is limited in some countries (Saito et al., 2003; Vaahtera et al., 2014; Vilcacundo et al., 2017).

Currently, there is an interest in the search for natural substances with antioxidant capacity to be used by the food and pharmaceutical industry. Lipid oxidation is named as lipid peroxidation with oxidation of unsaturated fatty acids of the membranes cell. In different studies, many methods to measure lipid peroxidation have been described. These methods are used directly to measure the antioxidant capacity of compounds (β-carotene bleaching assay, iodometric hydroperoxide measurement, Thiobarbituric Acid Reactive Substances (TBARS) assay, ultraviolet spectroscopic measurement of conjugated dienes methods). TBARS assay is a spectrophotometric method used to measure the lipid peroxidation for its easy methodology and low cost (Apak et al., 2016; Ghani et al., 2017; Espin et al., 2017; Laguerra et al., 2007).

TBA solution allowed identifying the MDA presence with the formation of the complex of pink color TBA-MDA. Other aldehydes are produced in lipid peroxidation. These aldehydes could also react to the TBA solution and absorb at 532 nm wavelength. Different studies have described antioxidant activity and inhibition of lipid peroxidation of isolated protein of animal and vegetable sources such as quinoa, rice, fish, egg, milk proteins, amaranth (*Amaranthus hypocondriacus* and *A. mantegazzianus*) and soybean (Allen &Wrieden, 1982; González-Montoya et al., 2018; Neves et al., 2017; Paucar-Menacho et al., 2017; Álvarez-Jubete et al., 2010).

The DCFH-DA fluorescence assay is a fast method used to detect and measure ROS production using *in vivo* animal models. In the last years, their use in the zebrafish embryos model has been described in the literature. It has been reported recently the use of an *in vivo* antioxidant method in Caco-

2 cells and zebrafish embryos using DCFH-DA (2,7-dichlorofluorescein diacetate), which is a nonionic substrate and nonpolar substrate crossing the cell membrane and is hydrolyzed enzymatically by intracellular esterase to the non-fluorescent DCFH. DCFH molecule is oxidized to the fluorescent dichlorofluorescein (DFC) product in the presence of ROS. For this reason, DFC fluorescence represents a measure of ROS in the cells. Kang et al., 2014 described ROS inhibition in zebrafish embryos with polysaccharide purified from aloe vera (*Aloe barbadensis*) gel using DCFH-DA as a fluorescent agent. Lee et al., 2013 reported the use of DCFH-DA for analysis of ROS inhibition in zebrafish embryos of fucoidan extracted from *Ecklonia cava* extract. Also, the anti-inflammatory activity of fucoidan extracted from *Ecklonia cava* was evaluated. LPS was used as inductor of inflammation in zebrafish embryos. Other authors report the use of DCFH-DA to inhibit ROS in cells. Goh et al., (2016), described ROS inhibition in human keratinocyte cell lines, HaCaT, and human monocytic cell lines, THP-1 from *Aronia melanocarpa* concentrate. Jensen et al., (2015) reported ROS inhibition in polymorphonuclear cells in an extract obtained of blue-green algae. Carrasco-Castilla et al., (2012) described the antioxidant activity of the protein hydrolysates evaluated using Caco-2 cell lines, for which the oxidative stress was induced by the addition of the free radical generator ABAP. ROS inhibition was determined with the DCFH-DA reactive. Chen et al., (2013) have described the evaluation of the anti-inflammatory effect of chalcone and chalcone analogues (compound 5 and 9), using fin amputation in the zebrafish larvae model. Fin amputation was carried out by cutting out half of the caudal fin. They found that the treatment with compound 9 not only affected wound-induced neutrophil recruitment, but also affected the Mpx enzymatic activity. Protein expression levels of pro-inflammatory factors (Mpx, NFκB, and TNFα) were also regulated by compound 9.

In the literature, food proteins and their hydrolysates have reported inhibition of lipid peroxidation. Protein concentrates and proteins isolate of animal and vegetal sources with biological properties such as antioxidant, anti-inflammatory, antinociceptive, antiviral, chemoprotective, antibacterial and antiulcerogenic activities, have been produced (Carrillo et al.,

2018a,b,c,d; Lara et al., 2017; Poveda et al., 2016; Rodríguez Saint-Jean et al., 2013; Toapanta et al., 2016; Vilcacundo et al., 2018a). Vilcacundo et al., (2017) have described a quinoa protein concentrate obtained from *Chenopodium quinoa* Willd with inhibition lipid peroxidation properties in the zebrafish larvae.

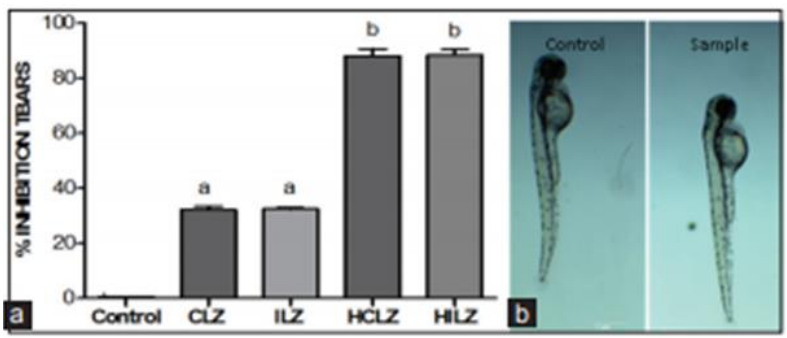

Figure 8. (a) Thiobarbituric acid reactive results of lysozyme. Data are expressed as % thiobarbituric acid reactive substances inhibition compared to a negative control (n=30 zebrafish larvae). Commercial lysozyme (CLZ), isolate lysozyme (ILZ), hydrolysate of CLZ and hydrolysate of ILZ, (b) Morphologies of zebrafish larvae after incubation with lysozymes (Carrillo et al., 2016a).

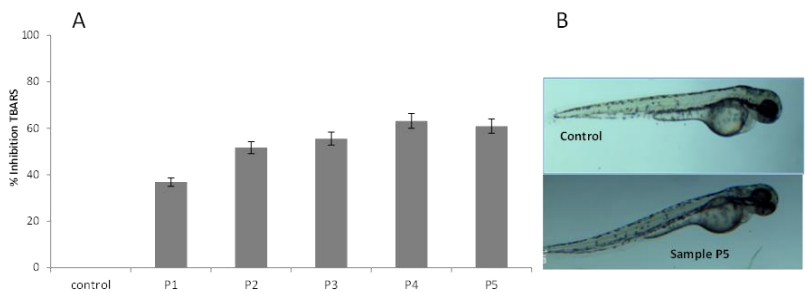

Figure 9. A) TBARS result of synthetic peptides from lysozyme. Data is expressed as % TBARS inhibition compared to positive control (error bars expressed as ± SD). P1= VAWRNRCKGTD; P2= IRGCRL; P3= WIRGCRL; P4= AWIRGCRL; P5= WRNRCKGTD. B) Photography of zebrafish larvae with peptide and without peptide after assay. All peptides were assay at 50 µg7ml (Carrillo et al., 2016b).

Carrillo et al., (2016b) have described hen egg white lysozyme (HEWL) isolate using a cation exchange chromatography and their hydrolysates with

inhibition lipid peroxidation properties in the zebrafish larvae model. HEWL was hydrolyzed with pepsin at pH 1.2 at 37 °C for 60 min. Commercial HEWL presented 21% of inhibition of lipid peroxidation and HEWL isolate presented 23% of inhibition of lipid peroxidation in zebrafish larvae. Commercial HEWL hydrolysate and HEWL isolate hydrolysate were able of inhibiting lipid peroxidation in zebrafish larvae with 82% and 84% respectively. Both HEWL and their hydrolysates present an absence of cytotoxicity in zebrafish embryos and larvae after 48 h of incubation.

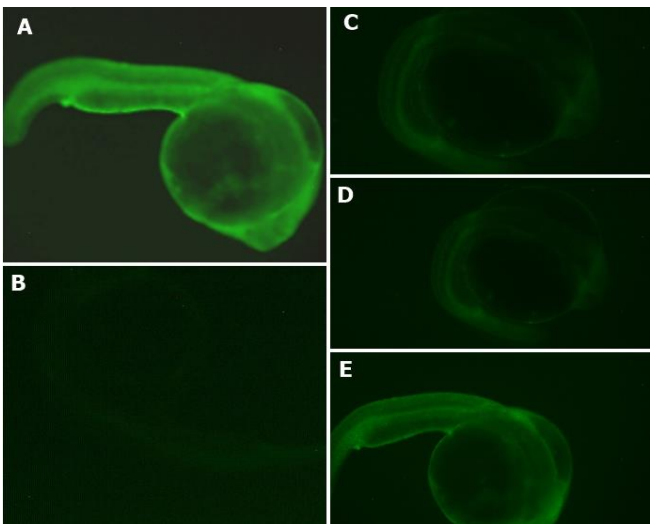

Figure 10. Effects of APC hydrolysates against AAPH-induced oxidative stress (ROS) in the zebrafish embryo model. A) AAPH treated group, B) Non-treated group, C) Gastric hydrolysate of APC at pH 2.0 and D) intestinal hydrolysate of APC and E) APC (amaranth protein concentrates without hydrolysis) (Vilcacundo et al., 2018).

Carrillo et al., (2016a) described the evaluation of inhibition of lipid peroxidation of five peptides identified of hen egg white lysozyme (HEWL). Five positively charged peptides were described: f(109-119) VAWRNRCKGTD with 36.8% of inhibition of lipid peroxidation, f(111-119) WRNRCKGTD with 51.6% inhibition, f(122-129) AWIRGCRL with 55.56% inhibition, f(123-129) WIRGCRL with 63.2% inhibition and f(124-129) IRGCRL with 61.0% inhibition, using the zebrafish larvae model.

Vilcacundo et al., (2018b) have described hydrolysates obtained of *Amaranthus caudatus* with inhibition lipid peroxidation properties and reduction of the formation of ROS in zebrafish embryos. The oxidation in zebrafish embryos was induced with AAPH. Then, the embryos were incubated with and without a sample together with the DCFH-DA (2,7-dichlorofluorescein diacetate). Hydrolysates from *Amaranthus caudatus* were able of reducing the intensity of fluorescence in the zebrafish embryos. Proteins concentrate without hydrolysis from *Amaranthus caudatus* present an absence of activity (Figure 10).

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Chapter 9

THERAPEUTIC MONITORING OF MIDAZOLAM: AN APPROACH TO ADEQUATE SEDATION IN CRITICALLY ILL PEDIATRIC PATIENTS

Carmen Flores Pérez*[*], Janett Flores Pérez, Gabriela Peña Morales, Karen Delgado Vergara and Hugo Juárez Olguín
Laboratorio de Farmacología, Instituto Nacional de Pediatría, Mexico City, Mexico

ABSTRACT

Midazolam (MDZ) is used for sedation in Pediatric Intensive Care Units (PICU) prior to any clinical procedure. However, management of sedation requires the assessment of its level using some clinical scoring tools. In Pediatric Intensive Care Units, the most common tools used for assessing sedation levels are the modified Ramsay and COMFORT scales.

[*] Corresponding Author Email: carmenfloresp@hotmail.com.

Nevertheless, the point against these two scales is their subjectiveness. In contrast, the electroencephalographic (EEG) methods such as audio-evoked potentials and Bispectral Index (BIS) objectively analyze the degree of consciousness. Bispectral Index is a continuous measurement of the level of consciousness by analyzing the frequencies of EEG waves and from there estimates the degree of brain electrical activity and the sedation of the patient.

The elimination of midazolam in PICU patients is low due to differences in age and disease status. Based on the possible existence of these variations, pharmacologists recommend that the best way to determine drug effect is to carry out therapeuric drug monitoring (TDM) to verify drug concentrations at different times. In view of this, TDM of midazolam was recommended, mainly to avoid the presence of adverse reactions.

In the Pediatric Intensive Care Unit of our hospital, it was found that with the brand of midazolam used, physicians have to apply higher infusion doses (6-folds higher) to reach the sedative status in children, with the risk of presenting adverse effects. This led us to conduct a study on the therapeutic efficacy of the two available brands of the drug – the innovator and the generic brands.

The study was conducted in twenty-six pediatric patients who were given initial doses of continuous infusion of 200 µg/kg/h, either from the innovator brand (Group A) or from the generic brand (Group B). The children were monitored for plasma concentrations determined by High Performance Liquid Chromatography (HPLC) and simultaneously, the sedative effect was measured using a BIS monitor. The results obtained were: *Group A*; Dose 284 (100-800) µg/kg/h, Plasma concentration (Cp) 725.3 (203.03 - 4633.04) ng/mL, BIS 72 (63 - 89), elimination half-time (t ½) 14.74 (2.1 - 56.7) h, and clearance (Cl) 3.04 (0.02-328.8) mL/h. *Group B*; Dose 500 (100-1200) µg/kg/h, Cp 4079.2 (694.4 - 7696.7) ng/ml, BIS 65.5 (42-82), t ½ 11.4 (1.1 - 57) h and Cl 1.29 (0.02 - 26.9) mL/h. Due to wide variation, the data are presented in medians and ranges.

To achieve an adequate sedation, the results showed that twice the dose of the generic brand is required when compared with group A. The variation in the result may be explained by the commercial characteristics of the generic brand. This chapter analyzes the possible causes leading to variations in the sedative effect of midazolam in children.

Keywords: Therapeutic drug monitoring, midazolam, sedation, critically ill children

INTRODUCTION

Midazolam is a drug that belongs to benzodiazepine family. Its clinical use was approved in 1976 as a hypnotic sedative and for the treatment of refractory crisis [1], as well as in the induction and maintenance of general anesthesia in order to achieve conscious sedation during diagnostic or therapeutic procedures [2].

To obtain an optimal level of sedation and analgesia is vital for the correct treatment of a critically ill child. The goal is to achieve a level of sedation and analgesia sufficient to eliminate pain, anxiety and agitation without causing secondary effects such as an excessive reduction of brain and/or respiratory activities, since inadequate sedation and analgesia can prolong mechanical ventilation and increase morbidity and mortality, as well as lengthen the time of admission in the pediatric intensive care unit (PICU) [3].

In the following topics, issues that are related to sedation of Midazolam in critical patients, such as the most commonly used sedation scales, pharmacokinetics-pharmacodynamics and the factors affecting them; main drug interactions and adverse effects will be addressed. Moreover, a therapeutic monitoring study recently carried out in the PICU of a pediatric hospital in Mexico City will be described.

SEDATION SCALES IN PEDIATRIC INTENSIVE THERAPY PATIENTS

The evaluation of the states of consciousness, sedation and analgesia is subjective and the available means for monitoring these states are scarce. The most commonly used methods to analyze the level of sedation are the clinical scoring scales that determine different physiological parameters. In children, the scales most commonly used are Ramsay scale [4] and the COMFORT scale [5] although, both are not very sensitive to the changes in deep sedation level.

Clinical Scoring Scales

The COMFORT scale is the only tool designed for use in children under mechanical ventilation. It consists of eight parameters, four physiological and four behavioral. Its evaluation requires several minutes (Table 1). The scale uses physiological parameters adapted to the age and does not require patient stimulation [6].

The scale is divided into three sedation ranges:

- 8 – 16: deep sedation
- 17 – 26: optimum sedation
- 27 – 40: inadequate sedation

One of the advantages of this scale is that it can be used in patients with mechanical ventilation, since the stimulation of the patient is not necessary**,** and requires only observation and the measurement of the physiological parameters. However, a point against the scale is the possibility of bias by different observers [7].

In previous studies where Bispectral Index (BIS) was compared with COMFORT scale in a paired group of ventilated and sedated children in PICU, the results showed a moderate correlation between the values of BIS and those of COMFORT. This is understandable because both measure different variables. The COMFORT scale permits the identification of patients with stress, agitation or discomfort while the BIS index measures the level of hypnosis according to an electroencephalographic pattern [6].

The Ramsay-Miller scale [8] is another clinical scoring tool. It is simple and fast and indicates the level of sedation of a patient (Table 2). Similar to COMFORT scale, this scale is divided into 3 sedation ranges:

- 0: no sedation
- 2 – 3: conscious sedation
- 4 – 6: deep sedation

Table 1. COMFORT Scale

Alertness:	
Deeply asleep	1
Lightly asleep	2
Drowsy	3
Fully awake and alert	4
Hyperalert	5
Calmness:	
Calm	1
Slightly anxious	2
Anxious	3
Very anxious	4
Panicky	5
Respiratory response:	
No coughing and no spontaneous respiration	1
Spontaneous respiration with little or no response to ventilation	2
Occasional coughing or resistance to ventilator	3
Actively breaths against ventilator or coughs regularly	4
Fights ventilator, cough or choking	5
Movement:	
No movement	1
Occasional slight movement	2
Frequent slight movement	3
Vigorous movement limited to extremities	4
Vigorous movements including torso and head	5
Muscle tone:	
Totally relaxed, no tone	1
Reduced tone	2
Normal tone	3
Increased tone with flexion of fingers and toes	4
Extreme rigidity and flexion of fingers and toes	5
Mean arterial pressure:	
Below baseline	1
Consistently at baseline	2
Infrequent elevations of ≥ 15% during observation period	3
Frequent elevation of ≥ 15% (> 3 during observation period)	4
Sustained elevation of ≥ 15%	5
Heart rate:	
Below baseline	1
Consistently at baseline	2
Infrequent elevations of ≥15% during observation period	3
Frequent elevation of ≥15% (>3 during observation period)	4
Sustained elevation of ≥15%	5
Facial expression:	
Facial muscles totally relaxed	1
Facial muscles with normal tone, no evident facial muscle tension	2
Tension evident in some facial muscles	3
Tension evident throughout facial muscles	4
Facial muscles contorted and grimacing	5

Table 2. Modified Ramsay-Miller scale

Level 1	Anxious, agitated, restless
Level 2	Eye opened, cooperative, oriented, tranquil
Level 3	Response (opens eyes) to commands, light touch, normal tone of voice
Level 4	Brisk response to light glabellar tap or loud voice/noise
Level 5	Sluggish response to light glabellar tap or loud voice/noise
Level 6	No response to light glabellar tap or loud voice/noise

The Ramsay scale (RS) was described and used since 1974 and although, it has undergone modifications over time, the essence of its evaluation remains the same. For this, it seems to be one of the most used in clinical setting. In countries of Europe where it is used, it is applied in up to 74% of the cases, because it has demonstrated an acceptable inter-observer reliability. Nevertheless, it has also been criticized for its lack of discrimination and specific descriptors that would allow those who use it to differentiate between the levels of sedation [9]. These drawbacks may be the reason why Jacobi et al. (2002) [10] do not propose it among the instruments to be used on daily basis. Regarding the frequency of its application, Covington pointed out that it should be used as many times as necessary, that is to say, every hour when the sedation level is evaluated or every 24 hours when the neurological status is evaluated in an integral manner [11].

Because of its simplicity, the Ramsay scale can be used by inexperienced professionals. It presents a great correlation with the evoked potentials when used to control sedated critically ill patients. However, it has several limitations such as lack of validation, inadequate assessment of agitation, non-mutually exclusive values, of limited use in patients with invasive mechanical ventilation and requires the stimulation and bothering of the patient when performing it [12]. In a comparative study of four different sedation scales aimed at determining their correlation with clinical data, it was demonstrated that Ramsay scale, not currently validated, has a strong relationship with other validated scales such as the sedation-agitation scale (SAS), Richmond agitation-sedation scale (RASS) and motor activity assessment scale (MAAS) [12].

Both Ramsay- Miller scale and the COMFORT scale are the most commonly used in pediatric ICU patients. The first for its simplicity and rapid assessment, is used in most cases, however, it has the disadvantage of requiring patient's response to verbal or physical stimuli, reason why it cannot be used in patients who are under mechanical ventilation. In such cases, it is advisable to use COMFORT scale that does not require the stimulation of the patient.

Bispectral Index (BIS)

In recent years, several methods that allow objective analysis of the degree of consciousness through encephalographic (EEG) determination have been developed. The most widely used are audio-evoked potential and Bispectral index (BIS) [13, 14]. The last estimates the degree of brain electrical activity and so, the sedation of the patient through the analysis of the frequencies of EEG waves (Synch-Fast-Slow = % fast frequencies/% slow frequencies) [14].

BIS is widely used in PICU because it is an objective and continuous measurement of the level of consciousness of a patient. EEG information is obtained through a sensor that is placed on the patient´s forehead. Its value can range from 0 to 100, zero in the case of complete EEG suppression and 100 in the fully awake patient (Figure 1) [15]. BIS monitoring has been validated as a measure of hypnosis in children older than 1 year and in adults. In children under 1 year, the available clinical experiences are few. Its use in all children is still incipient although, some studies have already demonstrated its usefulness during surgery [16, 17] and in patients admitted in PICU [18, 19].

In the intensive care setting, BIS monitor is usually used to achieve an objective assessment of sedation during different procedures (Table 3) [15].

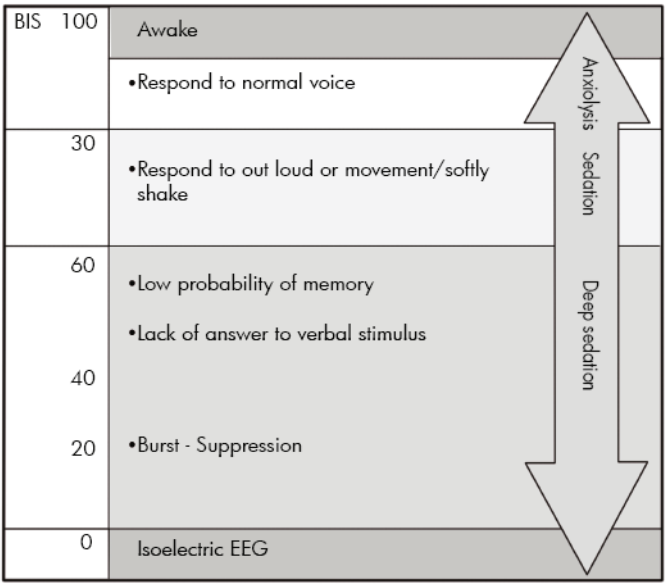

Figure 1. This chart reflects a general association between clinical state and BIS values. Ranges are based on results from a multi-center study of BIS monitoring involving the administration of specific anesthetic agents. BIS values and ranges assume that the EEG is free of artifacts that can affect its performance. Titration of anesthetics to BIS ranges should be dependent upon the individual goals established for each patient. These goals and associated BIS ranges may vary over time and in the context of patient status and treatment plan.

Table 3. Advantages of BIS in patients

Deep sedation for mehanical ventilation	• BIS can objectively measure the level of sedation facilitating the optimal sedative dosage to prevent over- or infra-sedation. • It can reduce clinical complications and costs due to faster weaning. • A better control of sedative dose can prevent the neccesity of using neuromuscular-blocker agents. • It allows the monitoring of intra-operative consciousness risk. • It affords the capacity to prevent adverse events associated with infra-sedation, for example, memory of unpleasant experiences.

Drug induced coma	• Nevertheless, BIS monitoring do not replace the complete diagnosis through EEG. The continuos objective information obtained simplifies the interpretation of patient's response and facilitates the dosage of drugs. • BIS monitor shows a continuous sketch of data from EEG. It calculates the percentage of time in the last minute of suppressed EEG signal, indicating the suppression rate. • The supression rate is related with burst-suppression and low BIS ranges. It continuously shows suppression rate in the monitor through a numerical value or as a tendency. • BIS value and suppression rate can be used as a dosing guide of sedatives agents, to mantain the coma induced by the drugs. • It allows a better adjustment of dose with continuous and objective information
Neuromuscular block	• The sedative evaluation scales are not effective in these patients. • It provides more information to assses the adequate dosage of drugs and to know the necessary drug type. • It ensures a low probability of explicit memory (BIS < 60) • It allows monitoring of intra-operative consciousness risk.
Procedures conduct In patient's bed	• The use of BIS allows the application of specific sedation doses to each patient, to avoid over- or infra-sedation during procedures in the ward, improving the quality of care and optimizing the sedation. • It allows the monitoring of intra-operative consciousness risk. • It allows the same quality care level compared with patients who received anesthesia and sedation in surgical procedures in the operating room. • It has potential savings in the costs of sedation and anasthesia.
Cares at the end of life	• The objective measures of conciousness level minimize the anxiety appearance and the possible unexpected recovery of patient conciousness.

The considerations of its use are that the doctor must make his judgment in conjunction with other available clinical signs whenever he interprets BIS; its readings must be interpreted with respect to time and in response to the stimuli, as well as the patient´s condition and the treatment plan. It should also be considered that with low BIS values, movement may occur, which is usually an indication of inadequate analgesia. It is not recommended to rely only on BIS values to determine the doses of sedatives. These should be interpreted with caution in patients suffering from neurologic disorders and in those who are being administered some type of psychoactive medications [20-24].

Regarding whether BIS is validated in ICU, it is know that most of the published data are related to its use in volunteers (early validation studies) and in patients in the operating room. The BIS values are directly related to other sedation assessment scales such as Ramsay scale [20, 25, 26], sedation-agitation scale (SAS) [20, 26, 27], Richmond sedation- agitation scale [28] and the COMFORT score [29, 30] that are commonly used.

PHARMACOKINETIC OF MIDAZOLAM IN CRITICALLY ILL CHILDREN

Depending on the patient´s clinical situation, the level of optimal sedation must be obtained at all times. Hence, it is essential to use the pharmacokinetic parameters which describe very closely what happens to the drug in the body once administered [31].

Absorption

In critical patients, midazolam is administered mainly intravenously (IV) and its bioavailability is 100% while intramuscularly (IM), it is 87% and orally (OV), it is 50%. The maximum serum concentration peak is reached in 15 minutes by IM route and in 53 minutes by oral route [32, 33].

Distribution

The volume of distribution (Vd) is from 1 to 3.1 L/k. 96% of the active drug circulates bond to proteins, mainly albumin, which promotes its distribution to the tissues and rapidly mobilizing it from the central nervous system to low perfusion tissues [32, 34].

Vd may be affected in critically ill patients with hypoproteinemia, since there is a decrease in protein binding with the drug. In such a case, there is an increase in the free form of the drug in the blood that is usually associated with a greater possibility of toxicity [32, 35].

Metabolism

Midazolam undergoes a biotransformation in the liver through the action of the cytochrome P450 3A4 (CYP3A4) in which the drug is changed to 1α-hydroxy-midazolam and 4-hydroxy-midazolam. 1α-hydroxy-midazolam represents 60 - 70% and has a 20% affinity to GABA, while 4-hydroxy-midazolam constitutes 5% or less and has approximately 7% affinity to the receptor [32, 36].

Excretion

The main route of excretion of the glucuronide-conjugated metabolites is the urinary route [32]. The excretion is affected in situations such as cardiac insufficiency, hepatic diseases and in some patients in critical state where glomerular filtration rate is reduced [37].

In normal conditions, the half-life ($t_{1/2}$) of midazolam is 2-3 hours, however, studies in critically ill patients have shown that the $t_{1/2}$ is longer due to poor metabolism and decreased clearance (Cl) [38, 39].

According to studies on pharmacokinetics of midazolam in ICU patients, variations in the serum concentration, distribution and elimination have been reported. Due to the variations in the effect and tachyphylaxis,

high concentrations of midazolam may be required, which increases the risk of presenting undesirable side effects in these patients [37, 40].

PHARMACODYNAMIC OF MIDAZOLAM

Gamma-aminobutyric acid (GABA) is a neurotransmitter with the most abundant inhibitory activity in the central nervous system [41]. GABA is stored in the pre-synaptic vesicles and thereafter, it is released by exocytosis. When this happens, it can bind to post-synaptic $GABA_A$ receptors. GABA has an inotropic receptor (the chlorine channel) denominated $GABA_A$, and the metabotropic receptor $GABA_B$ [42]. When two molecules of GABA are fixed in $GABA_A$ receptor, the opening of the chlorine channel occurs, which causes a hyperpolarization of the post synaptic cells responsible for the inhibition of the neurotransmission and clinically for sedation (Figure 2) [43].

GABA is usually fixed in two sites comprising of a subunit α and a subunit β and although, there are several types of subunits in the nervous system, these are related to different physiological and pharmacological functions [44].

The alpha subunits play an important role in the action of the benzodiazepines, since they are responsible for the pharmacological characteristics in terms of clinical and secondary effects [45]. Sedation, anterograde amnesia and anticonvulsant properties are mediated primarily by subunits α_1 of the GABA receptor. Anxiolysis and muscle relaxation are mainly mediated by subunits α_2 [46].

The existence of two types of benzodiazepine receptors has been reported: the central types (CBRs) which bind to the inotropic $GABA_A$ receptors and the peripheral types (PBRs) that do not bind to $GABA_A$ [47, 48]. Although, midazolam has shown to bind and to activate both receptors (CBRs and PBRs) [49], the sedative and hypnotic effects are mediated through the neuronal action on CBRs [2, 47, 50].

GABA-like benzodiazepine receptors in the spinal cord may play an important role in the analgesia, nevertheless, the mechanisms of action are not known in detail, since this could involve decreased excitatory synaptic transmission, direct action on the dorsal horn with an agonist effect of kappa and delta opioid receptors [51].

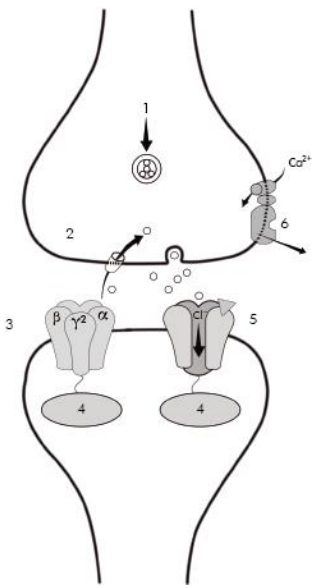

Figure 2. GABAergic synapsis. Gamma-aminobutyric acid (GABA) is stored in the presynaptic vesicles and later it is released by exocytosis. When it is released into the synaptic cleft, it binds to $GABA_A$ postsynaptic receptors among others. $GABA_A$ receptor is a pentameric macromolecular complex consisting of 5 subunits around a chloride channel. In the cytoplasm, $GABA_A$ receptors bind to a protein called Gefirine. 1. GABA; 2. GABA transporter; 3. $GABA_A$ receptor; 4. Gefirine; 5. Benzodiazepine; 6. $GABA_B$ receptor.

The peripheral fixation sites of benzodiazepines, other than those of GABA receptors have also been described [52]. These sites are present in many tissues, particularly in cardiovascular system, peripheral immune and digestive tube cells. Their functions are not known in detail, but they seem to be associated with mitochondrial metabolism with inhibitor effects on the

inflammatory mediators such as nitric oxide (NO), tumor necrosis factor α (TNF-α) and oxygen reactive species (ROS) [53].

Pharmacodynamic Studies of Midazolam

It is know that benzodiazepines play a role in the regulation of immune system [47, 54, 55]. In previous studies, it was found that prolonged intravenous administration of midazolam (more than 48 hours of continuous medication) can significantly affect immune function in elderly patients after surgery and inhibit the production of IL-1 β, IL-6 and TNF-α. Thus, it is highly recommended to monitor the immunological parameters in patients who are of advanced age, physically weak or immunocompromised that are undergoing treatment with midazolam [50].

Previous studies have shown that the expression of $GABA_A$ receptor-coupled CBRs in astrocytes can be altered during cell maturation, differentiation and activation [56]. While the expression of PBRs in normal human brain is very low, this expression increases in the astrocytes and micro glial cells following the occurrence of inflammation, brain injury, neurodegeneration and/or neuroglia in the glia [47, 48].

It is known that under physiological conditions, IL-1β and pro-inflammatory cytokines are expressed at low levels in the SNC. In traumatic brain injuries, cerebrovascular accidents and neurodegenerative diseases, the levels of IL-β increase in the cerebrospinal fluid and this can induce microglia and astrocytes to produce cytokines such as IL-6 and TFN-α [57-59].

In another study performed in pediatric patients after continuous intravenous administration of midazolam following a surgery, a trend towards a decrease in the immunological indices was found 48 hours after treatment, with a significant decrease in the levels of IL-1β, IL-6 and TNF-α when compared with the measurements before the treatment [59].

An anxiolytic and anti-depressive effect is obtained by a percentage of receptor binding less than 20%. An occupation of the receptors in the magnitude of 30-50% will produce sedative and amnesiac effects. The

hypnotic effect will present with values higher than 60% [43]. Based on the above mentioned in this section, it is important to considered that the intensity of the clinical effect is not only related to the degree of affinity of the drug with the receptors, but also to the doses administered. This must be taken into consideration when administering midazolam, since it may require dose adjustment to obtain the desired effect, thereby limiting the risk of overdose and other side effects on the nervous and immune systems.

FACTORS THAT ALTER THE PHARMACOKINETICS AND PHARMCODYNAMICS OF MIDAZOLAM

There are several factors, inherent in patients, that are involved in the metabolism of midazolam. The duration of the effects, the elimination time and the dose necessary to achieve the desired effect are aspects influenced by the presence of active metabolites, interaction with other drugs, metabolism of the medicine, patients premedicated with opioid analgesics, etc. [60].

The literature refers to certain diseases that can affect the pharmacokinetic of midazolam, reduce its Cl or prolong its $t_{1/2}$. It is likely that these effects are due to the decrease in the activity of CYP3A4 and CYP3A5 [61]. Alternative explanations could be an alteration in the binding protein, since midazolam plasma protein binding is high [62].

These same aspects are altered by the patient´s own characteristics such as age, nutritional status, (body weight), critical and inflammatory state (cancer, sepsis, multiorgan failure) and the presence of chronic diseases (nephropathies, hepatopathies, cardiopathies, pneumopathies, etc.) [63].

Identifying these concomitant physical and medical conditions woud minimize risk. Therefore, the patient should be assessed in order to identify what may increase or decrease the sensitivity to the anesthetic and sedative effects of midazolam. This would help to determine the adequate administration and dosage of the medication for each patient [64].

Age

Due to the differences observed in the expression and activity of CYP3A4 in liver and intestine in different age groups, it has been observed that the Cl of midazolam is lower in children compared to adults [65]. In children, the time for the clinical effect is greater for midazolam than for any other sedative agent [66].

Newborns have a reduced or immature organ function, so they are vulnerable to the deep and/or prolonged respiratory effects of midazolam. In these patients, the elimination $t_{1/2}$ is from 6-12 hours on average and the Cl is diminished [67].

Pediatric patients under 6 months of age are particularly vulnerable to the obstruction of the airways and to hypoventilation, therefore, it is essential to adjust the doses with small increment in function of the clinical effects and close control of the respiratory frequency and oxygen saturation [68].

In children of 3-10 years, the $t_{1/2}$ after the intravenous or rectal administration is shorter (1-15 hours) compared to that of adults. The difference is due to the elevated metabolic Cl in children of this age group [67].

The high metabolic rate observed in children compared to adolescents is explained by a decrease in the renal Cl of α-hydroxy-midazolam related with an early age [65].

The elderly have diminished liver function due to a decrease in the size of the liver and a reduction in the hepatic blood flow. The reduction in the metabolic capacity depends on the affected enzymatic system, which supposes an interindividual variability in the hepatic Cl. In adults older than 60 years, the $t_{1/2}$ can be extended up to four times. As a consequence of all these, the interactions are associated with more serious symptoms and have more important consequences than in the young population [67].

Nutritional Status

In patients with malnutrition, there is a decrease in plasma proteins, e.g., albumin which is responsible for the transport of many drugs including

midazolam. This situation leads to the alterations in the pharmacokinetic of this drug which, among others, produces a decrease in its Cl [69].

Midazolam is accumulated in the adipose tissue when it is administered in repeated doses. Hence, obese patients accumulate a greater amount of the drug and this increases the risk for significantly prolonged sedation effects [63]. The $t_{1/2}$ is longer in obese patients than in non-obese (5.9 hours compared with 2.3 hours) [67]. This is associated to an increase in the Vd observed in obese adolescents compared with normal weight adolescents. On the other hand, the difference in Cl between the obese and non-obese patients is not significant [70].

Chronic Diseases

Cardiopathies

Midazolam has myocardial depressant effect and produces a decrease in vascular resistance associated with the dose. The hemodynamic effects include a moderate decrease of the mean arterial pressure (15 to 20% with large doses), cardiac output and systolic volume. Thus, the use in patients with hemodynamic compromise is contraindicated [66]. In patients with heart failure, the $t_{1/2}$ is longer compared with healthy volunteers [67].

Pneumopathies

Midazolam produces depression of the respiratory center and depresses the response to CO_2, these being more evident in patients with chronic obstructive pulmonary diseases (COPD) [69].

Nephropathies

In patients with renal impairment (creatinine clearance < 10 ml/min), the pharmacokinetic (specifically the $t_{1/2}$) of free midazolam after a single intravenous dose is similar to that observed in healthy volunteers [71].

On the other hand, the metabolites, both those derived from their oxidation and those from their subsequent conjugation, are actives and can be accumulated in situations of renal failure [71]. After prolonged perfusion

in ICU patients, the mean duration of the sedative effect in population with renal insufficiency increased considerably, probably due to the accumulation of α-hydroximidazolam glucouronide [68].

Hepatopathies

Hepatic insufficiency reduces the Cl of intravenous midazolam with a subsequent increase in the $t_{1/2}$. Therefore, the clinical effects may be more potent and prolonged. In this case, it is pertinent to reduce the doses of midazolam and to establish adequate control of vital signs [68].

The $t_{1/2}$ in cirrhotic patients may be longer and the Cl lower compared to healthy volunteers [67].

Critical State

The pharmacokinetic parameters of midazolam of critically ill patients differ from healthy individuals due to pathophysiological alterations such as organ dysfunction or inflammatory condition that are frequently evident in critically ill patients [72].

From infancy to adolescence, critical sicknesses appear as the major determinant of midazolam Cl which results from the decrease of CYP3A4 activity associated to an inflammatory state. This can have important implications for the dosage of midazolam and other drug substrates of the CYP3A4 in critically ill pediatric patients [61].

In a study conducted in critically ill children with inflammatory effect, a decrease in the Cl of midazolam was observed [73]. In another comparative study in postoperative patients that received propofol or midazolam for 48 hours, a decrease in the pro-inflammatory cytokines (TNF, IL-1β, IL-6) was observed in patients who had received midazolam. The production of IL8 was also reduced [50].

Brussee et al. (2018) [74], reported a decrease in the Cl of midazolam when the concentrations of C-reactive proteins (CRP), that reflects the presence of inflammation, increased up to 3 times over its baseline without specifying the cause of the elevation. However, we believe that this could be

due to respiratory and cardiac disorders, sepsis and cardiac or non-cardiac surgery. There was also a 26% decrease in Cl depending on the severity of the sickness, expressed as the number of organs in dysfunction.

In a pilot study, it was suggested that the severity of a failure of an organ significantly correlates with midazolam Cl in critically ill children. Children with multiple organ failure have significantly reduced Cl compared with children without alterations in the organs. This is likely the result of the reduction of the activity of CYP3A [75].

In the case of oncological patients, a positive correlation between low albumin levels and a decrease in midazolam Cl was previously described. It was suggested that the reduction in the clearance is the result of a decrease in the protein binding. For this, Franken et al. (2017) [73] proposed that the mechanism behind the decrease in the clearance could be due to a base inflammatory response or a catabolic state. In cancer patients, hypoalbuminemia can be an expression of inflammation and it has been observation that this inflammation results from decreased activity of CYP3A.

Likewise, in another study in pediatric oncological patients, during acute inflammatory phase, a reduction in the Cl of midazolam was reported [74]. On the other hand, in a study performed by Barret et al. (2013) [76], five combinations of the drugs more commonly linked to toxicity in cancer patients were observed. The results suggested that one of these drugs simultaneously administered (e.g., Midazolam-fentanyl, midazolam-methotrexate, midazolam-vincristine, midazolam-lidocaine), may increase the risk of hepatotoxicity reflected in an increase in alanine-transferase (ALT) and a decrease of the neutrophil count.

As a consequence of the progress of their pathology, the patients with terminal disease are unable to respond adequately thus, obtaining higher scores in the Ramsay scale [77]. The effect of prolonged sedation with midazolam in critically ill patients may represent part of the spectrum of response and could be a reflection of the alterations of the liver flow or metabolic capacity [78]. In these patients, the $t_{1/2}$ of midazolam is up to six times longer [67].

Perez-Rada and cols. demonstrated that the associated complications such as delirium, sepsis, prolonged mechanical ventilation, longer hospital

stay and even mortality presented in a greater proportion in the group of patients who underwent surgery with midazolam [79].

When midazolam is administered for several days, its infusion should be progressively lowered to prevent the appearance of deprivation symptoms. The use of high doses could cause delay in awakening or unnecessary prolongation of mechanical ventilation time with its associated morbidity and mortality [71].

Considering the aforementioned, it is important to conclude that the dosage in clinical practice, above all; in hospitalized PICU patients in critical state due to oncological, infectious or chronic-degenerative processes**;** will require adjustment in time according to the progress of their disease or their basal condition to maintain effective concentration, avoid greater complications and reduce risk of adverse effects.

DRUG INTERACTIONS AND ADVERSE EFFECTS

Drug Interactions

Hospitalized patients who require sedation are usually medicated with other series of drugs that may influence in the pharmacokinetics or pharmacodynamics of midazolam, especially in critically ill patients who require different medications to achieve stability.

A very important group of these drugs are the inhibitors and inducers of CYP3A that have the potential of increasing or decreasing, respectively, the plasma concentrations and thus, the effects of midazolam.

Pharmacokinetic interactions with inhibitors or inducers of CYP3A4 are more pronounced with the oral administration of midazolam compared with the intravenous administration, specifically because CYP3A4 is also present in the upper part of gastrointestinal tract. This is because by oral route, both the systemic clearance and availability are altered while by the parenteral route, only the systemic clearance is altered [68].

CYP3A4 Inhibitory Drugs

Azole Antifungai

Ketoconazol, voriconazol, itraconazol and posaconazol increase up to 5 times the plasma concentrations of intravenous midazolam and up to 3 times its $t_{1/2}$. If administered parenterally, close monitoring should be carried out in case of respiratory depression and/or prolonged sedation. The staging and adjustment of the dose should be considered [80].

Macrolide Antibiotic

Erythromycin, clarithromycin and roxithromycin increase up to 2.5 times the plasma concentrations of midazolam and increase its $t_{1/2}$ in about 2 times [71].

HIV Protease Inhibitors

The concomitant administration with protease inhibitors may cause a large increase in the concentration of midazolam. The inhibition of CYP3A4 by ritonavir generates risk of serious toxicity with the administration of oral midazolam which leads in excessive or longer sedation, confusion, ataxia, weakness or cognitive or motor alteration [81].

Calcium Cannel Blockers

Verapamil and diltiazem increase the plasma concentrations of oral midazolam 3 and 4 times respectively. The $t_{1/2}$ of midazolam increased in 41% and 49% respectively [68].

Other Drugs and Food

Omeprazol can increase the plasma concentrations and the $t_{1/2}$ of the benzodiazepines including midazolam thus, increasing the risk of toxicity and decreasing its clearance. For the above, the administration of a benzodiazepine with lesser potential of interaction should be considered [82]. Gragefruit juice increases the bioavailability of some drugs by inhibiting CYP3A4 at the intestinal level. Some of the drugs seen to be more

affected by this interaction are some benzodiazepines such as midazolam [83].

CYP3A4 Inducing Drugs

Rifampicin decreased both intravenous and oral midazolam by 60% and 96% respectively. The $t_{1/2}$ was reduced in approximately 50% to 60% [71].

The administration of multiple doses of phenytoin or carbamazapine reduced the plasma concentration of oral midazolam by up to 90% and the $t_{1/2}$ in 60% [71].

Pharmacodynamic Drug Interactions

The concomitant administration of midazolam with other sedatives, hypnotics and CNS depressants, including alcohol, is likey to result in increased sedation and respiratory depression. Its simultaneous use with opioid derivatives, antipsychotics, other benzodiazepines, barbiturates, propofol, ketamine, etomidate, antidepressants, antihistaminics and antidepressive agents can also cause hypotension and prolong the recovery time of anesthesia [68].

In the case of some inhalation anesthetics, midazolam decreases the minimum alveolar concentrations of these drugs. It also reduces the minimum concentration of halothane necessary for a general anesthetic thus, it is necessary to use lesser doses of this anesthetic this drug when it is concomitantly administered with midazolam. When thiopental is used in the induction of anesthesia concomitant with midazolam, its doses should be reduced by 15% [68].

Santibañez et al. (2014) [84] conducted an observational study and found that morphine, fentanyl and chloral hydrate increase the central nervous system depressant effects either by central effect or by inhibition of midazolam metabolism. In another similar study, it was reported that the interaction between fentanyl, midazolam and propofol is associated with

bradycardia, hypotension and acute respiratory distress syndrome (ARDS) [85].

The principal drug interactions of midazolam based on the degree of interaction risk are shown below (Table 4) [86-88].

Table 4. Pharmacological interactions of Midazolam

Interaction risk	Drugs
Risk C *(Monitor therapy)*	Alcohol (ethyl), atorvastatin, bosentan, cannabis, chlorphenesin carbamate, CYP3A4 inducers (moderate): dexamethasone, nevirapine; CYP3A4 inhibitors (moderate): diltiazem, verapamil; dasatinib, deferasirox, doxylamine, fosaprepitant, *Ginkgo biloba*, hydroxyzine, magnesium sulfate, melatonin, mirtazapine, netupitant, palbociclib, propofol, sarilumab, selective serotonin reuptake inhibitors, siltuximab, simeprevir, tetrahydrocannabinol, tocilizumab, tofisopam, yohimbine.
Risk D *(Consider therapy modification)*	Buprenorphine, chlormethiazole, clozapine, CYP3A4 inducers (strong): carbamazepine, phenytoin, rifabutin, rifampin; dabrafenib, droperidol, enzalutamide, flunitrazepam, hydrocodone, macrolide antibiotics: azithromycin, erithromycin; methadone, mifepristone, mitotane, oxycodone, pitolisant, St John's wort, stiripentol, theophylline derivatives, zolpidem.
Risk X *(Avoid combination)*	Azelastine (nasal), boceprevir, cobicistat, conivaptan, fusidic acid (systemic), idelalisib, itraconazole, ketoconazole (systemic), olanzapine, orphenadrine, paraldehyde, protease inhibitors: indinavir, nelfinavir, ritonavir y saquinavir; telaprevir, thalidomide.

Adverse Effects

The following are the most common adverse effects reported for midazolam: hicuups, nausea, vomiting, laryngeal spasm, dyspnea, hallucination, dizziness, ataxia and involuntary movements. It also produces low blood pressure, low oxygen saturation and changes in heart and respiratory rhythm [89-91].

Marriot et al. (2004) [92] studied 117 patients undergoing conscious sedation under an established protocol and detected that adverse events occur in 35% (CI 95% = 27 - 44%) of the ptients, especially agitation (17.1%), hypotension (12.8%) and the associated hypoxemia (2.6%).

With overdose, ventilatory depression, apnea, areflexia, respiratory and/or cardiac arrest (usually in combination with central nervous system depressants) and episodes of hypotension may occur [91]. Nevertheless, in intensive care patients, the depression effect of CNS is a desired effect.

Table 5. Adverse effects of Midazolam

System	Adverse effects
Respiratory	Bradypnea (> 10%), decreased tidal volume (1% to 10%). Apnea (children: 3%), cough (1%) and dyspnea, hyperventilation, laryngospasm, bronchospasm and wheezing (< 1%)
Cardiovascular	Hypotension (children: 3%) and bigeminy, bradycardia, tachycardia, ventricular premature contractions (< 1%)
Central nervous system	Drowsiness (1%), headache (1%), seizure-like activity (children: 1%), drug dependence (physical and psychological dependence with prolonged use), myoclonus (preterm infants), severe sedation. Acidic taste, agitation, amnesia, confusion, delirium (emergence), euphoria, hallucination, sialorrhea (< 1%)
Gastrointestinal	Hiccups (adults: 4%; children: 1%), nausea (3%), vomiting (3%)
Skin and Teguments	Injection site reaction (IM: ≤ 4%, IV: ≤ 5%; severity less than diazepam), pain at injection site (IM: ≤ 4%, IV: ≤ 5%; severity less than diazepam) and skin rash (< 1%)
Ophthalmic	Nystagmus (children: 1%)
Miscellaneous	Paradoxical reaction (children: 2%)

The complications of insufficient sedation that have been reported are: fear, anxiety and agitation, risk of remembering unpleasant situations or being aware of them and the likelihood of removing medical devices unintentionally [27], while the complications of oversedation are: longer

periods of mechanical ventilation, longer stay in PICU and greater risk of complications related to concomitant diseases [93].

The principal adverse reactions that have been reported are mentioned in Table 5 [94-96].

In pediatric patients in critical condition, dose adjustment and continuous monitoring are important to reduce the risk of presenting the different adverse effects reported, since these patients usually require the drug for a prolonged time and need immediate attention in case of major undesirable effects.

THERAPEUTIC DRUG MONITORING

Currently, therapeutic drug monitoring (TDM) is an essential part of the patient´s care, since the drugs are intended to be efficacious and effective when administered in a specific therapeutic concentration. In other words, with the TDM, the exact dose of a medication to be prescribed can be estimated and in this way, maximize the benefits to the patients and minimize the risks of toxicity [97].

The advantage which can be obtained by the use of TDM is that when it is used appropriately and interpretation in a clinical and pharmacokinetic context, it turns out to be an excellent tool for the individualization of doses for patients [98].

MDZ Therapeutic Monitoring Study in a Pediatric Hospital in Mexico City

As mentioned, the pharmacokinetic of midazolam in PICU patients may be altered by simple or multiple organ failures. Likewise, these patients show a low clearance of the drug, due to differences in age and disease status [99]. So, it is advisable to carry out therapeutic monitoring study of the drug based on the determination of drug concentrations at different times and its relationship with sedative effect, to avoid the presence of adverse reactions.

Recently, we carried out a therapeutic monitoring study of midazolam in a pediatric intensive care unit, because the doctors observed that on applying midazolam ampoules of the generic brand, purchased in the hospital, at therapeutic doses of 200 μg/kg/h, patients were not completely sedated and required higher doses, up to 1200 μg/kg/h, to have them sedate with the possibility of presenting adverse effects. The study included 26 patients, aged 1-17 years old, without multiple organ failure, hepatic or renal insufficiency or any CNS diseases. The patients were given initial doses of continuous infusion of 200 μg/kg/h of midazolam either from the innovator brand (Group A) or the generic brand (Group B). Plasma levels of midazolam of the patients were determined by high-performance liquid chromatography (HPLC) and simultaneously, the levels of sedation in the patients were measured using BIS at different times: 1, 3, 12, 24 hours post-dose, 3 hours after dose reduction and 3 and 24 hours after the treatment was suspended.

Table 6. Results at 24 hours after the initiation of midazolam infusion in both treatment groups

Parameters	**Group A Median (range)**	**Group B Median (range)**
Demographic data		
Age (years)	8 (1 - 17)	10 (1 - 17)
BMI	15.8 (12.04 - 24.76)	19.13 (13.79 - 26.24)
Pharmacokinetics data		
Ke (h^{-1})	0.041 (0.012 - 0.32)	0.063 (0.012 - 0.623)
t ½ (h)	14.74 (2.1 - 56.7)	11.45 (1.1 - 57)
Vd (L)	6 (0.51 - 28.26)	2.1 (0.16 - 23.63)
Cl (mL/h)	3.04 (0.024 - 328.8)	1.29 (0.02 - 26.99)
MDZ concentration (ng/mL)	725.39 (203.034 - 4633.049)	4079.25 (694.448 - 7696.747)
Dose (μg/kg/h)	284 (100 - 800)	500 (100 - 1200)
Sedation level		
BIS Index	72 (63 - 89)	65.5 (42 - 82)

As can be observed in the results, almost twice the doses of the generic brand had to be administered to obtain an adequate sedation, with no toxicity or adverse reactions in both groups of patients.

The results of the pharmacokinetic parameters show values in their concentrations higher than those reported by other authors [99, 100]. In the first group, a slow elimination speed with an average $t_{1/2}$ of 15 hours is observed, while in the second group, although reaches higher concentrations, its elimination is faster with a $t_{1/2}$ of 12 hours. In terms of the Vd, there is an important difference since the first group shows a value almost three times greater than that of the second group and is reflected in the value of CI, which is greater in the first group.

Probably, the variability observed may be related to the characteristics of the formula, for example, the vehicle or the excipients used by the manufacturers. Likewise, as mentioned earlier in the section on *Factors that alter the pharmacokinetic and pharmacodynamic of midazolam*, there are several factors such as the age, nutritional status, inflammatory state, genetic factors, etc. that could influence sedation. All these put together could be the cause of the observed results.

To know the adequate dose of midazolam for this population and to avoid the presentation of toxicity and adverse reactions, we suggest in view of the above findings, that a study with a greater number of patients be carried out.

RECOMMENDATIONS

Throughout this chapter, the importance of obtaining the optimum level of sedation and analgesia in patients treated with midazolam in order to achieve the objective of its clinical and therapeutic use has been pointed out. This is particularly important for the correct treatment of critically ill children.

It is essential to consider the broad approach between the different assessment scales of sedation and the means available for their application, since these provide the necessary tools for patient monitoring, with the objective of maintaining an effective concentration that would translate to an adequate effect of the drug in patients subjected to different procedures in clinical practice.

The importance of comprehensive management during treatment with midazolam lies in the characteristics of each patient. The underlying disease and concomitant diseases, the totality of drugs that are coadministered and the factors related to the patient´s condition such as his surrounding environment and the seriousness of his disease must be considered. A greater attention must be paid to critically ill patients, since all these aspects alter and intervene directly in the pharmacokinetic and pharmacodynamic of the drug. The results obtained in the study carried out in our institution show that an adequate sedation was achieved through a close monitoring, taking into account the characteristic of the formulation of the drug applied, the doses used and the pharmacokinetic parameters as well as the wide range of physiological, pathological, immunological, genetic factors, etc. that are involved in the metabolism of midazolam.

Several studies that delve into the characteristics of this benzodiazepine from approaches such as its therapeutic use compared with other drugs with similar effects, pharmacovigeliance, drug interactions, posology, etc. abound in the literature. Nevertheless, there is still scarce information on its use in pediatric patients with critical illness in which the age and baseline status are related with a prolonged sedation and longer stay in the PICU. Hence, the contribution of this work is to fill up this information scarcity gap in the use of midazolam in the sedation of critically ill patient in the PICU.

Finally, it is a priority of this work to emphasize that the main objectives of adequate sedation in critically ill children are to reduce the risk of adverse effects and complications, avoid prolongation of the sedative effect and the time of stay in the ICU, achieve an individualization of the doses in order to get the desired effect and maintain each patient stable.

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BIOGRAPHICAL SKETCH

Carmen Flores Pérez

Affiliation: Researcher in Medical Sciences in National Institute of Pediatrics, Mexico City, Mexico

Education: Chemistry in Faculty of Chemistry at Universidad Nacional Autonoma de Mexico (UNAM), Mexico City. Management courses in Strategic planning in health and Clinical pharmacology, in Division of Postgraduate Studies from Faculty of Medicine at UNAM, Mexico City.

Research and Professional Experience: Member of National Researchers System in the period of 2005 to 2017. Experience of 19 years in the field of Pediatric Pharmacology, Professor of students under- and post-graduate in the fields of chemistry and medicine.

Publications from the Last 3 Years: 10 publications over 3 years

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