CURRENT ADVANCES IN MEDICINE - III

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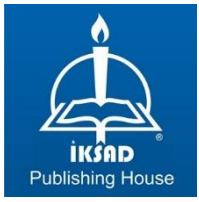

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Researches Publications®
(The Licence Number of Publicator: 2014/31220)
TURKEY TR: +90 342 606 06 75
USA: +1 631 685 0 853
E mail: iksadyayinevi@gmail.com
www.iksadyayinevi.com

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Iksad Publications – 2023©

ISBN: 978-625-6404-99-1
Cover Design: İbrahim KAYA
March / 2023
Ankara / Turkey
Size = 16x24 cm

CONTENTS

PREFACE

This book presents a diverse collection of topics that reflect the latest advances and best practices in medicine. The chapters included in this book are written by experts in their respective fields and provide a unique insight into the latest research and developments in medicine.

The topics covered in this book reflect a broad range of medical research and clinical practices, ranging from pediatric urology and cancer research to imaging technologies, wound care, and family medicine. Each chapter in this book explores a different area of medicine, providing readers with a comprehensive overview of the latest research and developments in the field.

We hope that this book will serve as a valuable resource for medical professionals, students, and researchers, providing them with the latest research and developments in the field of medicine. Our goal in compiling this book was to present readers with a broad overview of the latest advances and best practices in medicine, and we believe that the chapters included in this book reflect this goal.

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CHAPTER 1

ACUTE SCROTUM IN CHILDREN

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INTRODUCTION

Acute scrotum is used to describe sudden onset pain of the scrotum. Simultaneously, swelling and hyperemia in the scrotum may occur (Kass & Lundak, 1997; Tanaka et al., 2020). As will be explained later, although there are many causes of acute scrotum **(Table 1)**, testicular torsion should be considered in every patient presenting with pain and swelling in the scrotum and an evaluation should be made in this direction. Because the longer the torsion duration, the higher the risk of losing the vitality of the testis (Zhao et al., 2011). Differential diagnosis can often be made when a detailed history and physical examination are supported by an appropriate radiological imaging method.

Table 1: Causes of Acute Scrotum in Children

Pathology	Frequency	Age at presentation
Extravaginal torsion of testis	Uncommon	Perinatal
Intravaginal torsion of testis	Common	Anytime, peak at 13-16 yr
Testicular appendage torsion	Very common	Anytime, peak at 11 yr
Epididymitis	Rare	0-6 mo
Mumps orchitis	Uncommon	Only after puberty
Idiopathic scrotal edema	Uncommon	0-5 yr
Fat necrosis of scrotum	Rare	5-15 yr
Others*		
*** Henoch-Schönlein purpura, incarcerated inguinal hernia, hydrocele, varicocele, scrotal trauma, testicular tumor**		

1. TESTICULAR TORSION

Testicular torsion, which refers to the rotation of the testis around its vascular pedicle, is less common cause of acute scrotum, but it is the most important. It results in testicular loss if not promptly intervened.

The incidence of testicular torsion is 1/4000 in men under 25 years of age (Ringdahl & Teague, 2006). The most common age is neonatal and adolescence. Because the testis is attached to the surrounding tissues during its descent, the incidence of torsion is higher in undescended testicles. Torsion that occurs at the spermatic cord level outside the tunica vaginalis is referred

to as extravaginal torsion. Torsion seen in newborn or undescended testicles is often of this type. Almost all (95%) of torsion seen in newborn patients is intravaginal torsion. The cause of intravaginal torsion is the free movement of the testis in the scrotum due to an anomaly of adhesion of the tunica vaginalis to the epididymis and testis. This anomaly is called bell-clapper deformity (Prater & Overdorf, 1991). Although rare, torsion can be seen between the epididymis and testis.

In the etiology of testicular torsion, trauma, contractions of the cremaster muscles, cold, rapid growth and increased vascularity at puberty, iatrogenic torsion during surgery and undescended testis can be counted.

Testicular viability after testicular torsion is related to two parameters: duration and degree of torsion. The general opinion is that the first 6 (4-8) hours are very important **(Table 2).** However, it is stated in the literature that necrosis may develop in the first 2 hours, and necrosis may not occur at the end of 24 hours (Atallah et al., 1977). This situation is thought to be related to the degree of torsion. Since it cannot be known exactly when the testicle will lose its vitality; When torsion is diagnosed, immediate surgical intervention is necessary in all cases of testicular torsion.

Table 2: The relationship between torsion duration and testicular viability

Torsion duration (hours)	**Testicular survival rate (%)**
< 6	85 - 97
6 – 12	55 - 85
12 – 24	20 - 80
>24	**< 10**

Testicular Torsion in Neonates

This group accounts for 5-10% of all cases of testicular torsion.(Das & Singer, 1990). Torsion is usually extravaginal; testis, spermatic cord and tunica vaginalis were torsioned together. Neonatal torsions are divided into two groups; the first group is the prenatal (intrauterine) torsion group, the second group is the postnatal torsion group. Patients often present with a painless, dark, hard, non-transilluminating scrotal mass due to intrauterine torsion (Das & Singer, 1990). On ultrasonography, there is a mixed parenchymal echogenicity and calcification of the affected testis. There is no

blood flow in Doppler examination. In the differential diagnosis, meconium peritonitis, splenogonadal fusion and ectopic adrenal remnants should be kept in mind.

Treatment is planned based on the history. If the scrotum is normal in the early postpartum period but torsion findings develop in the later period, emergency surgery is indicated. However, there are two different opinions about the timing of surgery for prenatal torsions. The non-operative approach group who did not consider emergency surgical intervention necessary due to the possibility that the testis had already lost its vitality and that there was no bell-clepper deformity; The operative approach group recommending emergency surgery, saying that as the affected testis stays longer, it can affect the contralateral side, bell-clepper deformity cannot be excluded because bilateral cases are reported, and although rare, the distinction between teratoma and incarcerated hernia cannot be made clearly in newborns.

In conclusion, emergency surgical intervention should be performed due to the possibility of saving the testis in postnatal torsions and difficulty in differential diagnosis in prenatal torsions. In prenatal patients, an inguinal incision should be made, and fixation should be done by placing the testis in the subdartos pouch with a transverse scrotal incision.

Clinical Features

In most patients, the first symptom is sudden onset of unilateral scrotal pain and swelling. The pain is sometimes not severe and gradually increases in severity. This may cause delay in diagnosis. The pain may radiate to the inguinal region and even to the lower abdomen. Presence of similar pain in the history of some patients should suggest spontaneously reduced testicular torsion. This supports the diagnosis. The pain may begin at rest, while playing, or after trauma. Nausea and vomiting may accompany the pain (Gatti & Patrick Murphy, 2007).

Physical Examination

In the physical examination, the lower abdomen, inguinal region, penis, and scrotum should be carefully evaluated. Initially, the scrotal skin on the torsion side is hyperemic, edematous, and swollen. Severe pain on palpation is an indication that the testis has not gone into necrosis. Since the length of the spermatic cord is shortened due to torsion, the testis on the torsion side is located in the high scrotal position and is in a transverse

position. As edema and inflammation increase, normal testicular boundaries disappear. Cremasteric reflexes are lost on the torsion side, but the presence of reflexes does not rule out the diagnosis (Hutson, 2012).

In undescended testicular torsion, pain, hyperemia and swelling are in the inguinal region. Empty scrotum is significant in separating it from incarcerated hernia.

Intraabdominal testicular torsion has signs of acute abdomen.

In the differential diagnosis, epididymitis, epididymorchitis, orchitis, incarcerated hernia, testicular appendage torsion, acute hydrocele, traumatic hematoma or hematocele, idiopathic scrotal edema, scrotal fat necrosis, varicocele, testicular tumor, Henoch-Schonlein purpurtal infiltration, leukemia infiltrates should be kept in mind.

Complete blood count and urinalysis can be done in the laboratory evaluation. Although pyuria with bacteriuria is in favor of urinary tract infection, it does not exclude the diagnosis. Likewise, the presence or absence of white blood cell elevation is not a finding that excludes the diagnosis of testicular torsion.

Color Doppler USG is more helpful than conventional USG in acute scrotum. Arterial blood flow is reduced or absent on the testicular torsion side compared to the normal side. Observation of increased testicular blood flow excludes the diagnosis of torsion. However, it is not always possible to evaluate vascularity in young children (Baker et al., 2000).

Tc^{99m} pertechnetate scintigraphy is also used in the diagnosis of testicular torsion. Diagnostic accuracy increases to 95% in appropriate centers. However, the fact that the substance to be used in the examination is not always ready in every center prolongs the time for diagnosis. In addition, it has been reported that appropriate evaluation may not be made in small children due to the small size of the testicles (Wu et al., 2002).

It should be kept in mind that negative exploration is less risky than patients losing their testicles, and exploration should be performed without losing time in patients whose diagnosis cannot be confirmed.

Treatment

The duration of ischemia and the degree of torsion are the most important factors affecting necrosis.

Treatment is always surgical. Scrotal exploration has two purposes: Detorsion on the torsioned side and fixation on the opposite side to the scrotum.

Making the incision from the median raphe provides access to both testicles through a single incision. However, transverse incision over both hemiscrotum is also an accepted technique. After the torsioned testis is detorsioned, it is necessary to wait for the circulation to be monitored. While waiting, it is wrapped with a warm gauze compress. At this time, the contralateral testis is evaluated. If the blood supply of the torsioned side improves, orchiopexy should be performed on both testicles. The recommended method while performing orchiopexy is to open the tunica vaginalis, remove a part of the tunica vaginalis, and attach these edges to the external spermatic fascia with non-absorbable sutures within the dartos pouch. With this method, the testis can form a second mesoorchium by adhering to the scrotal tissues from the front and the fixation is strengthened. If the tunica is not to be removed, at least 3 points of fixation are required. Torsion can be seen after fixation with absorbable sutures. For this reason, even if there is a history of fixation, if there are signs of torsion in patients, torsion should be kept in mind in the differential diagnosis. Orchiectomy for testicles with circulatory disorders after detorsion is controversial. It has been shown in studies that ischemia disrupts the blood testicular barrier. It has also been determined that antibodies against their own spermatogonia are formed in patients older than 10 years. This has been confirmed by the fact that patients with fixed ischemic testicles have weak spermatogenesis in the future. Children under the age of 10 do not have the risk of autoimmunization because the blood testicular barrier is not formed. For this reason, some authors recommend leaving the ischemic testis in patients younger than 10 years of age and orchiectomy in patients older than 10 years of age (Hutson, 2012). As a result, there is no criterion to determine whether torsioned testicles can be left in place between 12-24 hours. However, there is a consensus on orchiectomy for testicles that remain ischemic for more than 24 hours. If there is undoubted necrosis and/or abscess in the torsioned testis, the tissues are debrided, a drain is placed, and antibiotic therapy is started. If there is a suspicion that the infection will spread to the opposite side, orchiopexy is left for another session.

Manual reduction over the scrotum can also be tried. However, this condition is not considered a definitive treatment. It can be tried in early stage

patients, in patients where surgery will be delayed due to anesthesia. Detorsion is tried to be achieved by turning the testis on the affected side laterally. Sudden relief of pain and inferior displacement of the testis indicates that the procedure was successful. Supporting with Doppler increases the success. Even if the detorsion is successful, both testicles should be fixed within a few days at the latest by surgical exploration (Gatti & Patrick Murphy, 2007).

Since 1/3 of the patients presenting with torsion have a history of intermittent recurrent testicular pain, elective bilateral orchiopexy is recommended for patients presenting with intermittent recurrent testicular pain (Hutson, 2012).

2. TESTICULAR APPENDAGE TORSION

Testicular appendage torsion is one of the leading causes of acute scrotum in children. Although it can be seen at any age, it is most common between the ages of 6-12. There are four appendages on the testis. The appendage testis is a Müllerian duct remnant, located in the upper pole of the testis, and is the most common (92%) structure with torsion. The appendage epididymis is the remnant of the Wolf's duct, located in the head of the epididymis and is the second most common (7%) structure with torsion. The other two structures are called appendage paradidymis (Giraldes organ) and appendage vas aberans.

Pain similar to testicular torsion is seen in testicular appendage torsion. However, the pain is not as severe as in testicular torsion. Pain and swelling gradually increase in a few days. Fever, nausea and vomiting are rare.

In the early stage, there is tenderness on palpation in the upper part of the testis. Torsioned appendage can be seen from the skin of the scrotum as a small discoloration. This is called the 'blue dot sign'. In the late stage, specific findings cannot be seen and the picture is similar to testicular torsion (Boettcher et al., 2012).

Radiologically, Doppler USG and nuclear scintigraphy can be performed. The blood flow may be the same in both testicles or may be increased due to inflammation on the affected side. Since the appendage cannot be seen on USG, the findings can be interpreted in favor of epididymitis (Laimer et al., 2022). However, testicular appendage torsion

should be considered primarily in patients with normal urinalysis and increased testicular blood flow in the pre-puberty period.

Bed rest, scrotal elevation and anti-inflammatory therapy are the first options for treatment. Surgery may be required in patients whose testicular torsion cannot be differentiated clearly or whose pain does not regress despite treatment.

3. EPIDIDYMITIS AND ORCHITIS

Epididymitis is infection and inflammation of the epididymis, which is often seen after puberty. Epididymitis before puberty is rare and often occurs due to urinary tract infection. If the patient's epididymitis is accompanied by urinary system infection or if epididymitis recurs, it should be evaluated for urinary system anomalies regardless of age. Ultrasonography and voiding cystourethrography are performed. The most common cause is vesicourethral reflux (Kadish & Bolte, 1998).

Orchitis is infection and inflammation of the testis. It usually occurs when the factor causing epididymitis spreads to the testis. For this reason, the term epididymo-orchitis is used. Orchitis alone is rarer. It occurs when an infection in another part of the body spreads by hematogenous route. Etiology includes mumps, scrotal trauma, epididymitis and systemic infections.

Mumps orchitis is usually seen in adolescents and is 80% unilateral. Testicular tenderness and stiffness are seen. It is accompanied by fever, dysuria and leukocytosis. Its treatment is scrotal elevation and use of analgesics. In 1/3 of the patients, the testis goes to atrophy (Wu et al., 2021).

In epididymitis, the pain is minimal at the beginning. Gradually, pain, edema, hyperemia increase. Initially, the pain is only in the infected epididymis. In the following period, the pain may spread to the testicle and inguinal region. These findings cause it to be confused with testicular torsion. Hydrocele may develop due to inflammation. The reduction of pain with elevation of the scrotum during the examination is called the 'Prehn's sign'. However, it is a nonspecific finding.

Laboratory evaluations may show pyuria and bacteriuria. A urine culture should also be obtained in patients with suspected epididymitis. E. coli is most commonly isolated. Doppler USG can be performed in radiological evaluation. Increased blood flow in the epididymis and testis is detected. Diffuse echo enhancement and often hydrocele are present.

Bed rest, scrotal elevation, analgesics, anti-inflammatory and antibiotics are recommended for treatment. Antibiotic treatment can be changed according to the culture result. Pain and edema are expected to regress in a week and induration within a few weeks (Somekh et al., 2004).

4. SCROTAL TRAUMA

Minor scrotal traumas are common in children. Prepubertal testicles are small and mobile. Therefore, serious injuries are rare. Compression between the pubic bones increases the risk of injury. Penetrating injuries are rare. Many different clinical conditions can occur as a result of scrotal trauma. Traumatic epididymitis occurs within a few days after trauma. Pain occurs at the time of trauma and quickly disappears. However, after a few days, gradually increasing pain occurs. On examination, the scrotum is hyperemic and edematous. Its treatment is conservative. In traumatic epididymal rupture, even if surgical intervention is performed, the results are not good.

In case of tunica albuginea laceration and intratesticular hematoma, drainage and tunica repair should be performed. Surgery may not be required if the amount of hematoma is small and the tunica is intact.

The accumulation of blood in the tunica vaginalis is called a hematocele. Because it is the result of severe trauma, there is usually ecchymosis and contusion on the scrotal skin. The diagnosis is supported by the fact that the fluid seen on USG is denser than the hydrocele fluid. Surgery is performed in case of excess fluid or testicular rupture. Surgical treatment accelerates recovery (Sadjo et al., 2021).

Testicular torsion can also be seen after any scrotal trauma. Findings and treatment are the same as for spontaneous testicular torsion.

The choice of surgical or conservative treatment after scrotal trauma should be chosen according to the clinical condition of the patients. Patients who are not treated surgically should be re-evaluated with USG after a few weeks.

5. OTHER CAUSES OF ACUTE SCROTUM

a. Acute Idiopathic Scrotal Edema

In acute idiopathic scrotal edema, there is sudden onset of edema and hyperemia. Pain is rare. Edema may extend to the penis and inguinal region. It usually occurs before puberty. The cause is unknown, but 60% of patients

have a history of allergy. It usually regresses within a few days with bed rest and scrotal elevation (Gatti & Patrick Murphy, 2007).

b. Henoch-Schönlein Purpura

Henoch-schönlein purpura is a systemic vasculitis with characteristic nonthrombocytopenic purpura, abdominal pain, arthralgia, gastrointestinal bleeding, microscopic hematuria, proteinuria, and nephritis. Vasculitis may also involve the spermatic cord and testis. In this case, it may cause testicular torsion-like pain and edema. This syndrome can be suspected if there are characteristic rashes and other findings. However, sometimes scrotal findings may precede systemic findings, making the diagnosis difficult. Even if Henoch-Schönlein purpura is suspected, it should be kept in mind that it may be associated with torsion (Gatti & Patrick Murphy, 2007).

c. Incarcerated Inguinal Hernia

It is the condition that the contents of the hernia do not return to the abdomen. It is seen in 12-17% of all childhood age groups. Incarceration is more common in the first age group (70%). Incarceration increases the risk of mortality (2-4%). If incarceration continues for a long time, lymphatic and venous flow is impaired, edema and congestion occur. With the deterioration of arterial blood flow, ischemia, gangrene and perforation occur. This is called strangulation.

Initially there may be restlessness, pain and vomiting. The pain increases over time. Redness and swelling of the skin are observed. Delayed patients may have vomiting, abdominal distention, inability to poop, inability to pass gas, and dehydration. Structures in the scrotum can be evaluated with USG. Gas shadows in both the intra-abdominal and scrotum can be evaluated on direct abdominal X-ray. Treatment is emergency surgery. The complication rate increased (5%). Manual reduction is not attempted in patients who arrive late. However, reduction with sedation can be attempted in patients who do not develop signs of ischemia. These patients should be operated 2-3 days after reduction for the regression of edema(Olesen et al., 2019).

d. Hydrocele

It is the filling of fluid into the "processus vaginalis". The amount of fluid may increase or decrease from time to time. Pain is not observed. Differential diagnosis can be made by transillumination (Alp et al., 2014).

e. Varicocele

It is used to describe the abnormal enlargement of the spermatic cord veins. There is a painless mass in the scrotum. It is usually asymptomatic and occurs between 10-15 years of age. It is mostly on the left. It is thought to be so because the left spermatic vein drains at a right angle to the renal vein. There is no change in the skin of the patients. The testis and epididymis are normally palpable. The veins are palpated as an enlarged mass above the testis. This is called the 'worm pack'. The patient is examined standing and lying down. Standing veins become more prominent. Varicocele may cause size difference in testicles over time. It is one of the causes of infertility in adults (Hutson, 2012).

f. Testicular Tumors

The clinical sign is painless unilateral swelling. Leukemic infiltration may be bilateral. Germ cell tumors are frequently seen (70-90%). Masses originating from yolk-sac and teratoma before puberty are more common. Local or diffuse increased hypervascular intratesticular mass is the typical finding on ultrasonography. The tumor is in the form of a focal hyperechoic or hypoechoic mass or a diffusely enlarged testis (Dicken & Billmire, 2012).

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CHAPTER 2

THE LOGIC OF PLATELET-RICH PLASMA

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INTRODUCTION

PRP is characterized as an autologous concentrated plasma fraction consisting of platelets in higher numbers that draw attention to its contents, e.g., cytokines and growth factors. Platelet concentration, platelet gel, and platelet lysate are additional terminology used in the literature to describe platelet-derived preparations. The goal of reparative and regenerative therapies is to supply at least one of the three essential elements - growth factor, scaffold, and cell - needed for tissue regeneration. PRP, on the other hand, might meet two components together with growth factor and scaffold. Its main advantage is that it would be obtained from autologous blood quickly and simply and applied in an abbreviated time. The use of PRP is considered an effective and convenient approach by different disciplines of medicine. However, the results from studies might seem contradictory because the preparation and application steps are not fully and distinctly standardized. However, it should be noted that if PRP-based products would be accepted as a live biomaterial and the studies on the content of PRP would be increased, the inconsistent clinical outcomes would be decreased. For this purpose, the process from platelets to the final product, that is, PRP, as well as PRP-based treatments, will be discussed in this section to improve clinical outcomes.

1. CELL STRUCTURE AND COMPONENTS OF PLATELETS

Platelets in the peripheral blood are discoid-shaped, anuclear, and approximately 2-3 μm in diameter and derived from the cytoplasm of megakaryocytes in the bone marrow (Textor, 2014). Megakaryocytes from bone marrow go through endomitosis, become polyploid, mature, and produce 1000-2000 platelets from their cytoplasm with numerous long projections called proplatelets. They constitute about 6% of the cells in the peripheral blood (93% erythrocytes, 6% platelets, 1% leukocytes) and the peripheral blood of humans contains $150–450\times10^9$ platelets/L. The life cycle in the peripheral blood takes about 10 days before being eliminated mainly from the liver via lectin receptors on the membrane surface (Mehta & Watson, 2008). In addition to the classification of the platelet structure as membrane/hyalomere/granulomere, platelets are now divided into the following physiological sections from another point of view (Arora et al., 2009; Cimmino & Golino, 2013; Thon & Italiano, 2012); i) Shell (cytoskeleton) or Peripheral Section: plasma membrane and open canalicular system (OCS). ii) Sol-gel Section: Central cytoplasm and actin network. iii)

Organelles Section: Granules, mitochondria, lysosome, peroxisome.

Platelets without a nucleus have a diffuse cytoskeleton, mitochondria, lysosomes, ribosomes, modified granular ER, unique organelles, and membrane structure. The platelet membrane serves a particularly specific function and contains complex invaginations, as demonstrated by electron microscopic examinations. These membranous invaginations, named the open canalicular system, extend into the cell and expand the surface area of the platelet membrane during activation. Following the activation of platelets, the cytoskeleton undergoes remodeling and develops pseudopodia. After the platelet granules are transported to the cell center and combined with the canalicular system through intracellular mechanisms such as vSNARE and tSNARE, the granule contents are released out of the cell (Blair & Flaumenhaft, 2009).

Another membranous component in the platelet cytoplasm is the dense tubular system, similar to the sarcoplasmic reticulum of muscle cells, which takes part in the sequestration of intracellular calcium when intracellular traffic is not intense (Rendu & Brohard-Bohn, 2001; White, 2007). The components of the intracellular skeleton include the circular microtubular ring, a prominent actin network, and a membrane skeleton. It has been shown that it might contract a hundred times more than myoblasts due to this developed actin network (Lam et al., 2011). The platelets come together in the primary hemostatic plug and get tightly packed and form a thrombus with this unique feature. The same feature is transmitted to the clot retraction process by the fibrin mesh in secondary hemostasis.

Platelets circulating close to the vessel wall are activated when they encounter stimuli such as subendothelial collagen, von Willebrand factor (vWF), thrombin, and ADP released in the damaged area. The result is combined cellular changes that are physical, chemical, or both (Rivera et al., 2009). Intracellular calcium increases and actin network remodeling occurs in activated platelets. Integrins are displaced in the membrane and allow platelet-fibrinogen binding. Thrombin is formed from the prothrombinase complex that appears on the membrane after activation via PAR receptors. Fibrin monomer is formed with fibrinogen, which acts as a substrate for thrombin. Subsequently, the coagulation cascade continues with the coagulation factors receptors appearing on the membrane. Platelets arriving at the damaged area are activated and continue cytokine and growth factor degranulation for 18 hours (Lindemann et al., 2001).

The granules in the platelet content are divided into alpha, dense, and lysosomal granules. Alpha granules are the uttermost diversified components, containing more than three hundred different proteins or active molecules (Coppinger et al., 2004; Van Nispen Tot Pannerden et al., 2010). A low level of protein synthesis in platelets is already known. In addition to the membrane bodies of dense and alpha granules and the platelet content being formed in megakaryocytes, a small portion of the granule content is also produced by uptake from plasma by both platelets and megakaryocytes (Kuter, 2012; Rendu & Brohard-Bohn, 2001). Growth factors are in zymogen form in alpha granules. The significance of the roles of these molecules in tissue healing and hemostasis has been reported by several researchers. The growth factors that are particularly emphasized in the studies are as follows: platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), transforming growth factor–beta (TGF-β), platelet factor 4, platelet-derived angiogenesis factor (PDAF), platelet-derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), vWF, osteonectin, osteocalcin, vitronectin, fibronectin, fibrinogen, thrombospondin-1, interleukin 1 (Arora et al., 2009; Eppley et al., 2006). The bone morphogenetic protein (BMP) family is thought to not be present in secretory granules, yet a recent study found BMP2 and BMP4 in platelet lysate (Eppley et al., 2006; Wahlström et al., 2008). The dense granules are comparatively rare and contain smaller molecules such as ATP, ADP, GTP, GDP, histamine, serotonin, magnesium, calcium, and polyphosphate (Rendu & Brohard-Bohn, 2001). Although lysosomes of platelets resemble those in other types of cells, their specific role has not been fully elucidated. Yet, they are considered to contribute to clot lysis (White, 2007).

2. HISTORY AND DESCRIPTION OF PLATELET-RICH PLASMA

In the study conducted in 1974 on atherosclerosis, the increase in proliferation of smooth muscle cells with the supplementation of 10% serum had a prominent place, however, it was not revealed which component of the serum provided this anabolic effect (Ross et al., 1974). It was assumed that the proliferative effect provided by the serum was caused by the thrombocytes. In 1978, Witte studied the degranulation of PDGF from human platelets, and in the ensuing year, Kaplan studied the localization of PDGF in alpha granules (Kaplan et al., 1979; Witte et al., 1978). In the following years,

studies on growth factors increased and the term "Regenerative Medicine" was mentioned for the first time by Kaiser in 1992, yet, the widespread use of this concept is attributed to William A. Haseltine, the founder of human genome sciences (Prasad, 2017). Diverse kinds of studies within the scope of regenerative medicine attempt to clarify the role of PRP in aiding and facilitating the healing process after injury. The possibility that autologous PRP does not trigger an immune response makes PRP usable by different disciplines of medicine (Alves & Grimalt, 2018). It is prepared with different protocols and used in private clinics as well as in hospitals. Since it is not categorized as a drug, the preparation and administration steps are not fully and clearly standardized, and therefore the effects of PRP seem to be contradictory. This inference has been reported in past studies investigating the cause of inconsistent clinical results (Hussain et al., 2017; Kramer & Keaney, 2018; Taylor et al., 2011).

3. PREPARATION AND ACTIVATION OF PLATELET-RICH PLASMA

The main goal of obtaining a PRP-based product is to establish a biomaterial with increased basal platelet values by centrifugation of anticoagulated whole blood. For this purpose, different methods have been discussed in the literature (Dhurat & Sukesh, 2014). It is essential to make PRP from anticoagulated blood therefore the platelets remain suspended and do not form clots, on the other hand, there is a loss of existing growth factors by pre-activation or lysis of platelets. There are even publications emphasizing the importance of the diameter of the needle used when taking peripheral blood (Arora et al., 2009; Lansdown & Fortier, 2017). PRP might also be diluted with platelet-poor plasma (PPP) according to the medical area in which it will be used.

Single or twice centrifugation (double spin) of whole blood might be conducted for the assemblage of the platelets. The double spin method has been used in the majority of the studies conducted to date. The coagulation is prevented by treating the whole blood of the patient with an anticoagulant such as acid citrate dextrose A (ACD-A), citrate, or ethylenediaminetetraacetic acid (EDTA). Mostly preferred is ACD-A or citrate. Anticoagulant-added blood might be stored for up to eight hours (Eppley et al., 2006).

It is common to apply a lighter translational force in the first centrifugation step of anticoagulant whole blood. Although it varies according to the centrifuge device, G force is recommended instead of RPM value because it is more standardized (Tekin et al., 2015). After the first centrifugation, the following three layers are expected to form in the tube: a transparent serum at the top, a red layer where the erythrocytes are concentrated at the bottom, and a darker layer than the serum called the "buffy coat" with platelets and leukocytes in the middle. After this stage, the second centrifugation process is continued by eliminating the bottom erythrocyte layer. For this, the uppermost serum layer and the lower “buffy coat” are transferred to the new flat tube with a pipette. For the upper two layers taken into the new tube, the second centrifugation is performed at a greater g-force. After the second rotation, platelet-poor plasma at the top and PRP at the bottom, that is, the pellet in which the platelets are concentrated accumulates in the tube. After the platelet-poor plasma is pipetted into a separate new tube, the remaining pellet forms the product to be used in PRP-based therapies (Muthu et al., 2022).

Activation of PRP, which is suggested to reveal the effects of PRP, is also one of the most crucial elements influencing the outcome of the treatment. Platelet activation, which is often needed for platelet degranulation and discharge of α granules into the pellet, is also a preferred procedure as it induces fibrinogen cleavage that promotes matrix formation (Akeda et al., 2019). To date, various endogenous and exogenous activation procedures have been used in experimental and clinical studies. These techniques are frequently performed by supplementing the pellet with calcium chloride or ADP or thrombin, applying freezing and thawing periods (>6 cycles) to the pellet, or exposing the pellet to tissue collagen (Akeda et al., 2019; Cavallo et al., 2016; Huber et al., 2016). While bovine thrombin is used in experimental studies, autologous thrombin should be prepared in human studies. Freezing and thawing cycles are considered a practical approach for in vitro studies as no additional ingredients are required. Some authors prefer in vivo contact of PRP with collagen after administration, as another form of activation. It has been suggested that this form of activation will increase the effectiveness of the PRP application by causing a slower and longer-lasting degranulation (Chalidis et al., 2023). However, if the tissue collagen is preferred as an activator since this step does not require a supplement such as calcium or thrombin, the possibility that the quality and amount of growth factors

released into the pellet might change should be considered.

As a result of differences in activation methods, the evaluation of outcomes might be contradictory. For this reason, while there are researchers who find it appropriate to use activated PRP, there are also researchers who prefer to use non-activated PRP (Gentile et al., 2017; Vahabi et al., 2018). Within ten minutes after activation, the greater part of the alpha granules begins to discharge rapidly (Marx, 2004). In the following hours, the contents of the remaining granules continue to be released. It is frequently encountered that PRP is used immediately after activation (Eppley et al., 2006), yet, some authors prefer to preserve PRP by storing it at low temperatures due to various clinical situations.

Today, application kits that provide sterile and practical application of these steps have been developed and there are various publications in the literature comparing them (Everts et al., 2006; Mazzucco et al., 2009). However, it should not be forgotten that the cost will increase if commercial kits are used to obtain PRP. As a result, inconsistency in the findings emerges as an inevitable result, as a different protocol may be applied for each step during the preparation phase. For this reason, reviewing the most up-to-date literature on the tissue or field of medicine that is planned to be used PRP before each administration.

4. RECENT STUDIES ON PLATELET-RICH PLASMA

Tissue repair after injury consists of overlapping phases: Phase 1. Hemostasis (1 to 3 days). Phase 2. Inflammation (3 to 20 days). Phase 3. Proliferation (1 to 6 weeks). Phase 4. Remodeling (6 weeks to 2 years). Each phase contains enhanced cellular and molecular activity. While platelets and blood plasma are attendants of hemostasis and clot formation, the activated platelets are also mediators of inflammation in conjunction with leukocytes. Platelets mediating the initiation of inflammation also contribute significantly to other wound-healing processes, including re-epithelialization, angiogenesis, and fibroplasia (Li et al., 2007). They provide a biological scaffold around the damaged area and become a reservoir of released bioactive molecules.

Numerous studies have reported that growth factors released from platelets play key roles in the acceleration and facilitation of injury response and the stimulation of the formation of extracellular matrix and connective tissue elements (Luttenberger et al., 2000). While the primary role of platelets is hemostasis, their secondary task is to release growth factors and chemo-

attractants and initiate the healing process. As a result of the activation, the platelet content is discharged into the microenvironment. Various growth factors released, particularly anabolic ones, such as EGF, IGF-1, PDGF, VEGF, FGF, and HGF, contribute to wound repair and tissue regeneration. Leukocyte infiltration occurs due to the degranulation of platelet-derived chemotactic factors, and the leukocyte-contaminated PRP is believed to affect the inflammatory stage in this way. The fact that leukocytes will play a significant antimicrobial role, especially in cases susceptible to infection, makes leukocyte contamination acceptable in PRP. For example, a past study has reported a significant reduction in chest wound infections and reduced drainage of wounds in the leg and chest regions following coronary artery bypass surgery with the application of LR-PRP. Yet, more elucidative studies are needed to directly compare the effect of LR-PRP with LP-PRP (Wasterlain et al., 2012).

Angiogenesis and mitogenesis-promoting growth factors are esteemed to contribute to wound healing and tissue regeneration. The pre-synthesized growth factors begin to be actively released within ten minutes from the onset of coagulation, and over 95% of these proteins are released within the following hour. Released proteins bind to surrounding target cells, such as mesenchymal cells, osteoblasts, fibroblasts, and epidermal and endothelial cells. Through these components, platelets participate in the inflammatory-proliferation-re-modeling steps of wound healing, cellular proliferation and migration (IGF-1, PDGF, FGF, EGF, TGF-b, HGH), differentiation, matrix synthesis (TGF-b), angiogenesis (VEGF, Angiopoietin-1, HGF, CD40L, TGF-B1, and PDGF). Bioactive substances have a direct effect on the cellular environment and micro-environment in the area they are released, therefore, both the formulation and composition of PRP are among the factors that affect the overall improvement. Therefore, the response from PRP is influenced by the method of obtaining, the number of white blood cells as well as the number of platelets, and the concentrations of fibrinogen, fibrin, and growth factors. Therefore, it is essential to characterize the cellular and molecular components of PRP-based treatments when evaluating their efficacy (Barrientos et al., 2008; Kang et al., 2011; Nurden, 2011; Sánchez-González et al., 2012).

Maintaining proper organization of the extracellular matrix to support performance and increase functionality in intact tissue is one of the most desirable goals in regenerative medicine. To this end, the studies examining the integrated effects of serotonin, platelet-derived growth factors, matrix metalloproteinases, and matrix metalloproteinase tissue inhibitors on the production and remodeling of the extracellular matrix provide guidance (Nurden, 2011). In healing wounds, fibroblasts are directed towards the fibrin clot by the chemotaxis-stimulating effect induced by TGF-β and PDGF (Dees et al., 2011). These cells also migrate along the fibronectin line stimulated by platelets. In addition to TGF-β, fibroblasts are stimulated to synthesize collagen and fibronectin with the contribution of serotonin derived from platelets. Platelets not only stimulate matrix synthesis but also induce cell proliferation and differentiation (Kakudo et al., 2008; Mishra et al., 2009; Wang et al., 2012).

PDGF, one of the secretory proteins released, has widespread clinical use. As an FDA-approved recombinant PDGF product, Becaplermin is used topically on chronic wounds such as diabetic ulcers (Bolton, 2016; Papanas & Maltezos, 2010). Experimental studies on the tendon, cartilage, joint healing, and periodontal alveolar reconstruction are ongoing. In addition to these, platelets also show a direct vasoconstrictor effect with the serotonin they contain. Leukocyte activation and B cell transformation occur with the chemokines and cytokines secreted. Studies on the antimicrobial and antifungal protein content of platelets, like primary immune cells, are available in the literature (Cieślik-Bielecka et al., 2018; Nguyen et al., 2011; Speth et al., 2014).

There is increasing evidence that platelets and their activation states can modulate immune responses. The use of low platelet count as an indicator of poor prognosis in septic patients supports this view. Another convincing evidence was provided by a study reporting that platelets killed malaria in infected erythrocytes (McMorran et al., 2009). The role of platelets in the proinflammatory process has been demonstrated in certain disorders or diseases, such as rheumatoid arthritis, atherosclerosis, multiple sclerosis, and transfusion-associated acute lung injury. In addition to being proinflammatory, platelets also exert an anti-inflammatory response by blocking the signaling pathway of the nuclear factor kappa B (NF-κβ) in the target cell and with its tissue metalloproteinase inhibitor component (Murate et al., 1997; Santos-Martínez et al., 2008). The combined effect of platelet

growth factors, serotonin, matrix metalloproteinases, and TIMPs in the construction and reorganization of the extracellular matrix has already been the subject of previous studies.

Studies within the scope of orthopedics draw attention to the necessity of TGF-β for cartilage matrix homeostasis and internal repair. Moreover, a considerable number of publications also emphasize the necessity of TGF-β for chondrogenic induction of mesenchymal stem cells. However, the use of TGF-β as an intraarticular injectable therapeutic agent is under investigation due to its fibrogenic effects (Grässel & Aszódi, 2017). Similar to BMP, a member of the TGF-β superfamily, TGF-β stimulates matrix synthesis and proliferation in osteoblasts and acts as a negative regulator by inhibiting the release of receptor-activator nuclear factor kappa beta ligand (RANKL) (Chen et al., 2012). The opportunity to choose desirable effects on matrix production and composition without inducing pathological fibrogenesis will be provided by the precise therapeutic use of TGF- β as a unique standalone agent or as a PRP ingredient.

Both experimental and therapeutic studies on the in vivo and in vitro use of PRP are increasing day by day. Studies that include curative results in maxillofacial surgery have been pioneers in this regard (Marx et al., 1998). As a final result of the progress in treatment with PRP, the repair-enhancing effect of PRP in periodontal implants is widely accepted (Arora et al., 2010; Del Fabbro et al., 2011). Currently, the positive effects of PRP on humans continue to be examined from different perspectives by various disciplines. The disciplines and related fields that address this issue include skin ulcers such as bedsores and diabetic ulcers, plastic reconstruction and cosmetic surgery, oral-maxillofacial surgery, sports injuries, (especially cartilage-tendon-joint repair), orthopedic surgery-bone reconstruction (ischemic osteonecrosis, malunion, osteolysis, musculoskeletal diseases), ophthalmological problems (corneal ulcers), dermatology (especially alopecia), neurosurgery, urology, and cardiovascular surgery (Bastami et al., 2017; Gentile et al., 2017; Kon & Filardo, 2011; Martinez-Zapata et al., 2016; Tambella et al., 2018).

Due to its anabolic properties and capacity to promote the differentiation of chondrocytes and tenocytes, PRP is frequently used in orthopedic diseases. Early in the new millennium, PRP injections were being researched as a potential osteoarthritis treatment. In various investigations, the effectiveness of treatment with PRP intra-articular injections into joints with

osteoarthritis has been compared with hyaluronic acid. In terms of both pain level and function of the joint, PRP injections in osteoarthritis promise a potential therapy for the patients (Ferneini et al., 2022). Intra-articular PRP injections for patients with osteoarthritis offer results that compete with hyaluronic acid. PRP injections promise an effective therapy in terms of both pain level and joint movement. In a study that included a minimum 14-month follow-up of patients with symptomatic knee osteoarthritis, patients with moderate disease scores benefited greatly from intra-articular PRP application, however, those with severe clinical course benefited less from the injections, according to the Visual Analogue Scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores (Annaniemi et al., 2023). However, in another study on osteoarthritis, either PRP, glucocorticoid, or hyaluronic acid was injected into the knees of patients as a single agent. As a result of the study, no decrease in pain level or regression in secondary parameters such as WOMAC score was detected in the short or long term in any of the patients with mild and moderate knee osteoarthritis. However, it is noteworthy that the median age of the patients in this study was 60. It will be important to compare the results of a similar study with a younger patient population, because perhaps PRP treatment, which will be applied in the first years of symptoms, will provide clinical improvement (Tschopp et al., 2022).

The intense interest in cosmetology allows PRP to be tested in this field as well. PRP, with its high regenerative capacity, emerges with an innovative approach as an effective and non-invasive method for facial rejuvenation treatment against signs of aging. Apart from the use of PRP alone, there are also clinical studies in which it is used as a supplement with additional substances. In a recent study, a case with pemphigus vulgaris and deep gingival recession who did not respond to additional steroid and immunosuppressant treatments achieved complete remission with repeated Ritukimab and PRP applications (Gabusi et al., 2023).

In another recent study, limbal autograft application was performed on patients suffering from total unilateral limbal stem cell deficiency due to chemical burns, accompanied by autologous PRP drops, and very successful results were obtained in corneal re-epithelialization (Baradaran-Rafii et al., 2022). PRP, which is widely used in different clinics for eye diseases, has also been evaluated in patients presenting with unresponsive persistent neurotrophic epithelial defects. In vivo confocal microscopy and optical

coherence tomography analyzes were performed in these patients, accompanied by clinical evaluations based on various parameters and scores such as corneal esthesiometry, corneal healing time, and visual acuity. As a result of a four-month observation, researchers determined that the application of PRP with fibrin glue could heal the epithelial defects of the patients in the first ten days (Baradaran-Rafii et al., 2022).

Another feature of platelets is that they contribute to the formation of new tissue both in the case of disease and in the absence of disease. As a result of the studies carried out to shed light on infertility cases accompanied by platelet dysfunction, it has been revealed that platelets play a direct role in granulosa cell luteinization and induce corpus luteum growth with angiogenesis stimulation (Basini et al., 2018).

CONCLUSION

Treatment with PRP concentrates is thought to promote the repair process following the acute injury or accelerate healing in chronic wounds. The tissue healing cascade includes platelets, leukocytes, fibrin matrix, and degranulated growth factors and cytokines. The therapeutic applicability of PRP is based on the demand of accumulating the active compounds (proteins, growth factors, lysosomes, cytokines, small molecules) to initiate and facilitate the formation of connective tissue elements, the revascularization, wound healing, the reparation and regeneration, and rejuvenation. Biomaterials containing platelet-poor plasma also contain plasma proteins (eg fibrinogen, fibronectin, prothrombin). Since the synergistic effect is provided by the interaction of the bioactive factors that PRP contains at supraphysiological levels with the microenvironment, there is a need for connections to be established between the PRP content and the paracrine, autocrine, endocrine, and intracrine interactions that occur in the tissue. Following platelet activation, the coagulation cascade continues with the release of dense contents of α-granules, polymerization of fibrinogen (released by platelets or free in plasma) into a fibrin network, and formation of a platelet plug. For this reason, there is a tendency for researchers to use activated-PRP. Besides all these, there are no clear regulations regarding a standard PRP preparation protocol. Therefore, PRP concentrates might differ in terms of platelet content, as well as white blood cell count or red blood cell contamination. These constitute solid evidence to recognize that PRP is heterogeneous and to predict that the effects of PRP might be more complex than that of conventional pharmaceuticals. Knowing that the materials

obtained from PRP differ in terms of components and therefore interact with the local microenvironment of the recipient will help us understand the diversity of clinical outcomes of PRP applications. As a result, PRP-based products should be considered living biomaterials and related studies should be customized for the PRP content and the tissue to be treated.

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CHAPTER 3

THE JOURNEY FROM LAB DATA TO BIG DATA

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INTRODUCTION

The new trend in health services is shifting from the disease-centered to the patient-centered model, from the paternalistic physician-patient relationship to an equitable partnership, and from experience to data-based evidence. (Newell & Jordan, 2015). It is obvious that different computational tools and methods should be used in order to process and finalize such complex and large data. Big Data is also called “the power of data”. On the other hand, big data in health refers to various and complex data that are difficult to analyze and manage with traditional software or hardware (Galetsi & Katsaliaki 2020; Raghupathi & Raghupathi 2014). This dataset is utilized to guide decisions about symptomatic patient care, epidemiological tendencies, resource allotment and encompasses extensive clinical, diagnostic, demographic and public health record datasets.

The characteristics of big data are defined by 6 V as Value (value), Volume (size), Velocity (speed of data access), Variety (variability in the format of the data), Veracity (the accuracy of the data) and Variability (the variability of the data) (Archenaa & Anita, 2015). The concepts of "Big Data Analytics" were introduced in order to systematically process and interpret rapidly growing data. Big data analytics encompasses the integration of heterogeneous data, data quality control, analysis, modelling, interpretation and validation (Wu et al.;2016). The big data analytics application provides comprehensive information discovered from the vast amount of data available. Big data analytics in medicine and healthcare combines many scientific analyzes such as bioinformatics, medical imaging, sensor informatics, medical informatics and health informatics. Analysis of big data enables the analysis of large datasets of thousands of patients, determines the correlation between clusters and datasets, and also develops predictive models using data mining techniques (Viceconti et al.;2015).

It may facilitate to predict or individualize the signs that allow early diagnosis of possible diseases by examining the clinical histories of patients who develop similar pathologies in particular. Recently, concepts and techniques such as computational medicine, computational pathology, computational medical laboratory, personalized medicine artificial intelligent and machine learning have become used in daily routine together with big data. Big data processing with machine learning and artificial intelligence is growing like an avalanche in the clinical laboratory field. Information technologies such as cloud computing and internet of things (IoT), empirical

technologies and procedures, sensor technology (wearable devices) and social networks boost use of big data. The main application areas of big data in health are resource optimization, electronic health records (EHRs), real-time alerting, increasing patient participation, use of health data for strategic planning, cancer treatment, predictive and preventive analytics in healthcare, "tele-medicine", integration of big data, unnecessary emergency prevention of service referral and therapeutic personalization (Nayak et al.;2016). Although the popularity of big data and related studies have grown intensively, there are some difficulties with data integration, storage, analysis and interpretation of results. Some of these are shown in **Table 1**, in short, poor predictive performance is due to the lack of causal link and clinical validation. There are some problems with big data specific to laboratory test results. These are mainly lack of standardization in test nomenclature, differences in units of measurement, differences in analytical quality (method-dependent results, lack of traceability), and use of different reference intervals.

Table 1: Challenges in Big Data Analytics.

• **Lack of clinical practice validation** **– Internal validation** **– External validation** **– Confirmation by prospective clinical studies**
• Difficulties in model interpretation – Classical hypothesis-experiment-no flow of results – Only correlation and statistical relationship available – No mechanistic link or causality
• **Insufficient predictive performance for clinical application** **– Heterogeneous data** **– Missing data** **– Garbage data** **– Inappropriateness of selected samples** **– Variability and noise of techniques**

1. DATA PRIVACY AND SECURITY IN BIG DATA

Personalized medicine promises to revolutionize healthcare. The success of this revolution depends on the accessibility of the health and biomedical data to the scientific community. It is essential for patients to agree sharing their personal and health data in this context. It is clear that patient data sharing is a critical process. Ethical, social, legal and technological challenges also arise while data sharing offers unprecedented

opportunities in precision medicine, Data sharing cannot be accomplished without the need for data security and privacy (Leff & Yang, 2015). Overcoming these barriers requires an interdisciplinary effort from computational scientists, physicians, patient advocates, regulators and health insurance providers. There is a need to better manage expectations and concerns (somewhat unrealistic) about data science and AI-based solutions. In this context, it should be noted that no algorithm replaces a doctor but rather provides them with a tool to support their decisions on the basis of objective, data-based criteria and the wealth of biomedical information available. Different aspects should be considered when defining data sharing. It is especially important whether data sharing includes individual data, data access period and duration along with the data which will be shared (Taichman et al.;2017). Overall, restrictions on data sharing remain a controversial issue throughout biomedical research, as demonstrated in recent discussions (Longo & Drazen, 2016;Buck et al.;2016). A recent study highlights data privacy and security as major sources ofpublic concern regarding biobanking and data sharing in general (Majumder et al.;2016). Anonymization or deidentification, encryption algorithms and authorization of all users (authentication) are major methods to provide security.

2. ELECTRONIC HEALTH RECORDS (EHR)

EHR consists of genetic testing, “omics” data, point-of-care testing (POCT), drug information, wearable devices, and literature information **(Table 2)**. EHR data is increasing 48% per year with a 15 times multiplication in 7 years.

Table 2: Data that make up Electronic Health Records.

• **Laboratory tests**
• Medical.images – Ultrasound – Magnetic.resonance imaging – Computerized.tomography
• Clinical notes
• Diagnoses
• Administrative data

• Tables
• Charts
• Prescriptions
• **Procedures**

Previously unknown clinical relationships will be learned, poorly designed and expensive clinical studies will decrease therefore costs will decline with the big data obtained from EHR. The budget spent on health services is around 6-11% globally, and the loss due to operational inefficiencies in health is $430 billion. It is thought that 330 billion dollars of this can be saved. The use of the EHR will not have the limitations of considering the small sample size and few confounders in randomized control studies (RCTs) because the EHR includes virtually all information recorded during clinical practice in contrast to only a few can be considered confounders in RCTs. EHR clinical database sample size enables including and assessing more variables in multivariate regression with a response vector matrix while methods such as multiple regression with a number of predictors are used to evaluate confounder effect in RCTs.

3. PERSONALIZED MEDICINE (PRECISION MEDICINE)

Personalized medicine is a new approach to disease prevention and treatment based on each patient's specific genetics, environment and lifestyle choices. The vision of personalized medicine is to provide the right treatment to the right patient at the right time and in the right dose. The goal of personalized medicine is to understand and identify biomarkers of specific diseases in order to provide patients with precise and individualized disease treatment, to ensure optimal therapeutic effects and to minimize damage and medical costs. Recent advances in high-throughput omics technologies allow to generate large volumes of biomedical data for personalized medicine (Merelli et al., 2014). AI-based systems can identify individual drug-response variability, makeArecommendations based on patterns learned from multiple public and private data sources, and help expand the genomic frontier of personalized medicine and cancer in particular (Lin & Kuo, 2018).

Some advantages of personalized medicine (Mathur & Sutton, 2017; Vogenberg et al.;2010);

- Ensuring a higher drug efficacy as treatments are tailored to patient characteristics such as genetic profile.
- Reducing the risks of adverse events by avoiding treatments that do not have a significant positive effect on the disease but also have side effects.
- Lower healthcare costs
- Early disease diagnosis and prevention using biomarkers
- Improvement of disease management through wearable sensors and mobile health applications, etc.

4. ARTIFICIAL INTELLIGENCE AND ITS USE IN MEDICINE

Jeopardy and AlphaGo are well-known human-competing artificial intelligence applications. Self-driving cars, smart homes, chatbots, retail marketing and high-volume commerce are other examples. Integration of artificial intelligence (AI) and machine learning (ML) with cloud technologies and EHRs expands the potential of personalized medical data and physician notes are extracted with Natural Language processing (NLP) (17). There are deep learning (Deep Learning, DL) and ML applications related to imaging. AI has performed as well as experts in diagnosing metastatic breast cancer, melanoma and various eye diseases. AI applications plays an important role in areas such as gene editing, CRISPR and drug discovery (Bedi et al;2015).

AI is a combination of theories, algorithms and computational frameworks that facilitate various tasks that require human intelligence such as reasoning, decision making, speech recognition, language understanding, and visual perception. It is a term that includes numerous methods such as logic learning (rule-based), machine learning, deep learning, NLP, and computer vision. AI will greatly assist us in the processing and interpretation of big data. AI speeds up the process of analyzing huge amounts of data, exploits patterns in data and enables faster and better recommended decision making. Advanced prediction models are created using algorithms that extract patterns from data and predict results. The greater the availability of data in a domain, the greater the adoption and use of such Disruptive Technologies. The emergence of big data with its increasing storage volume and computing power is pushing us towards making data meaningful and

actionable predictions rather than collecting data. Personalized medicine shows high promise only with the help of advanced algorithms derived from data sciences especially machine learning as data volumes increase while storage and processing costs fall. Modern machine learning algorithms have the potential to integrate multiscale, multimodal, and longitudinal patient data to make relatively accurate predictions that in some instances even exceed human performance. Major commercial players entering the field of medicine today underline the widely noticed potential for their computational solutions.

5. ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING IN MEDICAL LABORATORIES

New data needs to be assessed with a fresh mindset to use AI and ML methods because of multi-dimensional and expanding nature of the data. Although laboratory data does not take up much space on computers, medical laboratories are the leaders of big data in EHR when the transaction volume is taken into account. Adoption of AI and ML methods in medical laboratories lately are quite expeditious (Abadi et al.;2017) **Table 3** shows the usage areas of AI and ML methods in medical laboratories in which they can be used in different areas from workflow management to quality control and auto-verification.

Table 3: Big Data Analytics, AI and ML use in medical laboratories

Better utilization of Non-Laboratory Electronic Health Records
• Reducing laboratory errors
• Optimizing processes
• Facilitating test ordering with artificial intelligence and machine learning
• Creation of diagnostic algorithms with AI and ML
• Determination of reference ranges from hospital data
• Monitoring of quality indicators
• On-line monitoring of Calibration and Quality Control data
• Patient Based Quality Control using real time data
• Real-time workload analysis
• Reduction of lead times
• On-line monitoring of preventive maintenance-repairs of devices
• Predictive quality control applications
• Resource tracking
• Reducing cost and workforce

6. WHAT WILL BE THE PLACE OF MEDICAL LABORATORIES IN THE DIGITAL AGE?

Industrial manufacturing, economy and social life have been rapidly revolutionized by digital abilities in recent years. Health utility digitization

will have an overall effect in medicine particularly in laboratory medicine. Modern technologies will substantially revise the laboratories with regards to interest, policy and perception in the future and accumulating test results from a variety of sources about patient status will be enabled. Wearable equipment and body-implanted sensors will facilitate obtaining health data which will/can be stored in cloud services. These changes will facilitate Laboratory Medicine techniques and concepts to move forward and reach a higher level of discernibility which in turn reformulates laboratory relationship of doctor-patient as a diagnostic knowledge hub other. Laboratory Medicine will obtain digital health data in broader terms and this will enhance medical communication.

New educational concepts and continuing professional development will be needed in health data digitization to adapt and improve the methods we conduct our profession. “Creative destruction” is a technical term defined by the AustrianAeconomist Joseph SchumpeterAin macroeconomics in the early 1940s (Zhang, 2017). It is based on the assumption that when an existing system is toppled, it has the potential to be replaced by a new one.

Triggers are leaps in technology that typically act as disruptors (Wen et al.;2022). Increasing complexity between destructive events improves existing technologies until the next cycle of a creative destruction. The series of industrial revolutions that began with the steam engine in the 18th century is a prime example of Schumpeter's hypothesis (Ab Rahman et al.;2017). Traditional medical laboratories play a role in advising and helping clinician in diagnosis and follow-up. Therefore, the clinician is the leading actor. Usually doctors assess diagnostic results but the lab is behind the scene in the overall operation so lab processes are barely noticeable by the patients. Nonetheless, the focus of modern medicine is on the patient rather than disease. Medical Laboratories can assume more responsibilities in the future as a "nerve center of diagnostics". The concept of the "Diagnostics 4.0" triangle (patient-physician-laboratory) should be constructed by arbitrating the physician and the patient (Neumaier, 2019; Ceriotti, 2019).

Laboratory Medicine must proactively act to take its place in such developments. History has a tendency to repeat itself. Laboratory tests performed at the doctor's office indicually and then progressed towards more capable and extensive private/hospital laboratories at the beginning of the 20th century. Economic production was targeted with automation capabilities in technology and laboratories started to be automated and centralized in the

second half of the 20th century. Distributed laboratories are now an option thanks to new capabilities, which enable patients wearing sensors on them, point of care testing facilities and other enhancements in instrument technologies on the contrary to historic development of central Laboratory Medicine (Plebani, 2018).

Centralization and decentralization eras occur according to Plebani's interpretation of Giambattista Vico's recurrent loop theory for clinical laboratory (Plebani, 2018). Will clinicians and laboratory experts necessarily be replaced if patients carry out their own tests and obtain results with ML algorithms based on mobile and/or web applications? These are the issues being arisen regularly today. However, medical laboratories and their experts will continue to play an important role until a disruptive technology emerges to replace them. The justification for this are summarized in **Table 4**. Analytical competence in diagnostic tests will transform into the ability of data consolidation interpretation within clinical ambiance concurrently since the health data is plenty.

Laboratory diagnostic efficiency will increase with more clear medical explanations achieved through intense dialogue/consultation of physician and patient in the clinical decision-making process.

Table 4: Without a "Disruptive Technology" laboratory medicine and reasons for the survival of his expertise

Poor quality of existing big data
• Differences in current measurement technologies
• Lack of standardization among existing measurement methods
• Differences in traceability of existing methods
• Differences in repeatability and accuracy of existing methods
• Units of the same test are given differently between laboratories.
• Differences in reference ranges
• High quality POCT systems for multiple test analysis
technical problems in the development
• POCT costs
• No "lab-on-a-chip" yet !!!
• Ethical and legal problems
• Mandatory verification of applications and diagnostic algorithms

7. TRANSFORMATION OF MEDICAL LABORATORY EXPERTISE IN DIAGNOSTICS 4.0

Laboratory expertise is a product of incorporating analytical and clinical knowledge. It is not an easy task to achieve such experties in current laboratory operations since most of the clinical data originates from patient on demand tests. The ability of reaching real-time information enables laboratories to achieve disease prevention and early treatment. Health services are expected to get better with uninterrupted monitoring devices and applications. In case of tunica albuginea laceration and intratesticular hematoma, drainage and tunica repair should be performed.

Laboratory medicine will be confronted with new issues such as sample timing, outcome clarification and managing pre-analytical and analytical steps after laboratory test data is fused with EHR of several sources. Data flows from various origins will only be convenient if they are compatible. New quality evaluation methods are required for medical laboratories to face challenges of electronic health (eHealth) and mobile (mHealth) concepts of Diagnostic 4.0 Medicine has fallen behind in digitization because physician-patient confidentiality demands unconditional preservation of all data to review and transmit outside the physician's office. These issues are investigated in current digital era but patients are more open about it. Current generations post their identity relevant information quite easily to online platforms so whether they will be willing or not to share their health information is a matter of discussion. Patients and physicians need to adapt themselves to the new mechanisms. Modern countries have strict laws and legal arrangements to protect patient rights, enforce doctor-patient confidentialy and provide medicine and controlled substance safety.

That's said, imposing rigid patient right protection for environments outside medical domain in which personal health data is gathered, stored and governed is quite unattainable. The emergence of a set of more flexible legal rules are expected to form in the future for health data similar to "consumer protection rights". It can be briefly said that prospective laboratory medicine is an area of significance for healthcare professionals (Neumaier, 2019; Ceriotti, 2019).

Clinical laboratory will maintain its major position in laboratory medicine in Digital Health age. Superior intuition about DNA sequence and disease association shall be contributed by leading genome projects. Clinical

laboratories will progress into a more functional role in translational medicine, cutting-edge technology, clinical information management and control of the quality of results obtained outside the laboratory (**Table 5**). The two concurrent processes seem to execute together. The first one is the traditional laboratory testing unification is the former while POCT market expansion is the latter. The fast advancements in information technologies will improve consulting aspect of clinical laboratories more by expediting remote control of POCT analyzers and straightforward contact with patients (Plebani, 2018).

Table 5: Where will laboratory medicine focus in the digital age?

-Implementation of total quality management
• Ensuring effective diagnostic management
– Clinical efficacy
– PatientAsafety and risk management
– Outputs
– Operational efficiency
• Focusing on the value it generates, not the test volume
• Consolidation in traditional testing
• Quality control
– Focus on "Accuracy Medicine" alongside personalized medicine
• Reducing inappropriate test requests
• Reducing laboratory errors
• Consolidation of routine tests
• Ensuring global standardization of tests
• Finding new tests
• Management of POCT (Point of Care Testing) and "Near-Patient Tests"
– Approval of technology
- Quality assurance
- Education
- Consultancy
• Verification of test interpretation algorithms
• Integration from the stuck "Silo model" to the clinic and the patient
– Clinical communication: Consultation and counseling

As a result;

- A strong computational infrastructure is required for big data.
- New tools such as AI and ML should be utilized for the rapid and effective use of the huge growing data.
- Artificial Intelligence should now be inside the lab.
- It should be open to interdisciplinary studies within and outside the institution.

- “Computational Laboratory Medicine” units should be established.
- It should not be forgotten that the future is in the Learning Health System & Learning Laboratories.

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CHAPTER 4

WOUND CARE IN PLASTIC SURGERY AND PREPARATION OF THE WOUND SITE FOR SURGICAL PROCEDURES

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INTRODUCTION

Wounds are one of the most important health problems faced by human beings since the ancient times. (Brocke, T .et al;2020-7) Plastic surgery clinics train physicians in line with reconstructive principles. They are the main branch of medicine dealing with the causes and treatment of the wound.

Wound can be defined simply as deterioration in the structure of the tissue. Skin wounds are the most common wounds that we plastic surgeons encounter. Many different etiologies can be encountered in wound formation. (Forrest R. D. Et al;1982-22)

We frequently encounter injuries from traffic accidents, firearms, sharp objects, falls, animal bites, and burns. (Ramasastry S. S. Et al;2005-48) Also, the presence of cancer and chronic diseases causes us to encounter different wound processes. Plastic surgery consultations from emergency services are mostly due to the acute wounds. (Korting, H. C et al;2011-32) In outpatient clinics and inpatient services, plastic surgeons are frequently consulted for wounds in the subacute/chronic process.

Regardless of the underlying cause, all kinds of dirty, contaminated, necrotic and clean tissue defects of different widths and depths formed in the skin and the lower layers formed in the acute, subacute or chronic process are called wounds. (Raziyeva, K et al;2021-50)

The loss of tissue integrity that occurs at the time of injury causes us to encounter the picture of acute injury. Considering the etiological source of the injury, traumatic wounds can often be clean as well as dirty contaminated to cause infection. Since they cause thermal damage in firearm injuries, we often encounter clean but additional tissue damage caused by burns. Penetrating wounds are often a source of contamination. Burns are considered clean wounds, especially in the early period which can become infected later due to loss of integrity in the skin. However, secondary infections may occur with the disappearance of the protective barrier, as there is a loss of integrity in the skin with the advancing process in burn wounds. Animal or human bites should be considered infected and the treatment process should continue with this in mind.

Wounds that require prolonged treatment are considered subacute, while those with a history of several weeks or more are considered chronic.

(Ramasastry S. S. Et al;1998-47) Wounds developed on the basis of chronic diseases such as ischemic or rheumatic diseases are good examples of chronic wounds. In particular, distal lower extremity wounds of diabetic patients that develop due to peripheral vascular complications such as atherosclerosis and microangiopathies which cause ischemia, are among the most resistant wound types that we encounter in clinics. Pressure sores are the most troublesome group of wounds we encounter in bedridden patients in poor general condition and in need of care. Skin lesions of malignant diseases are good examples of chronic wounds, whether primary or metastatic.

1. WOUND HEALING PHYSIOLOGY

Wound healing is a dynamic, biological, complex process consisting of four interlocking phases in which different cells, cytokines and growth hormones play an active role. The aim is to ensure that the damaged tissue heals as close to its original state. (Rodrigues, M et al;2019-52) (Wysocki A. B. Et al;1999-64)

Cytokines and growth factors play an important role in intercellular communication. (Werner, S.et al;2003-62) Cells communicate with each other thanks to substances in protein structure that play a role in this communication with many chemotactic properties such as IL-1,IL-2,PDGF,FGF,TGF-BETA,IGF,EGF,GM-CSF. In this way, it is aimed to eliminate the damage by providing cellular proliferation, angiogenesis and extracellular matrix formation at the point where the damage occurs. (Castaño, O et al;2018-9)

Wound healing consists of four consecutive phases, namely hemostasis, inflammation, proliferation, and maturation/remodeling. The active role in the hemostasis phase is in the thrombocyte cells. They play a role in the formation of clots, which come into contact with the collagen in the damaged vessel wall and act as a plug. Inflammatory cells such as neutrophils, monocytes and macrophages play a role in the 72-96 hours following the first 24-hour period. Necrotic debris and bacteria are destroyed in this phase and various cytokines and growth factors are released intensively. (Knighton, D. R et al;1991-30) As angiogenesis and granulation tissue formation become evident on the 4th-5th day following the injury, the wound bed begins to be fed better and tissue oxygenation begins to increase. (Tonnesen, M. G et al;2000-59) After the 5th day until the end of the second week, the process of

approximately 10 days is considered as the proliferation phase. With the active role of fibroblasts in this phase, epithelialization progressing through the granulation tissue in the wound can be easily noticed. (Clark R. A. Et al;1993-12) A reduction in wound size occurs with the contraction effect of myofibroblasts.

At the end of the two-week healing process, the fourth phase, remodeling, begins and is considered a phase that lasts for years. In this phase, also called the maturation phase, collagen fibers are reshaped. The more unstable type 3 collagen is replaced by the firmer and more durable type 1 collagen. During the first six-week period of this phase, the wound reaches 95% of its original tensile strength. Any pause and prolongation in the phases of wound healing causes us to encounter hypertrophic and keloidal scar formations. (Wilkinson, H. N et al;2020-63)

2. FACTORS AFFECTING WOUND HEALING

Many systemic and local factors have positive or negative effects on existing wound healing. Examples of local factors include the presence of foreign body in the tissue, the presence of necrotic tissue, local infection in the surrounding soft tissues, dryness of the wound and insufficient moistening. (Nuutila, K et al;2021-41) Systemic factors have a wide spectrum, and any disease that may impair the patient's hemodynamic balance, wound healing capacity, systemic oxygenation, nutrition and anabolic process can be counted among them. (Brown, S. A et al;2004-8)

It would be correct to give diabetes mellitus as one of the most significant examples of systemic diseases. There is almost no system that it does not adversely affect. It creates damage at both macro and microangiopathic levels and has negative consequences on wound healing. HIV+ patients with immunosuppression who undergo chemotherapy due to malignant diseases are the patient groups with suppressed immune system. Such patients become susceptible to infections and wound healing processes are interrupted by indirect effects. (Ren, S et al;2022-51)

People with rheumatic diseases may experience negative effects on their wound healing capacity due to medical treatments such as systemic steroid use. Radiotherapy is an oncological treatment that can cause burn-like tissue damage and impair local wound healing, leading to tissue destruction.

The effect of this treatment method, which is frequently applied especially in the treatment of breast cancer, of damage to local tissues lasts for months.

3. WOUND CHARACTERISTICS

Many wounds, large and small, in different localizations of the body, resulting from various causes, appear in our medical life. The causes and localizations may be different, but the target is the same for all wounds. The main goal of plastic surgery is to make the wound infection-free, clean and ready for the occlusive surgical procedure. (Daeschlein G. Et al;2013-13)

The presence of any foreign body in the wound increases the risk of local infection. All foreign bodies that can be reached surgically must be removed.

All tissues that have lost their vitality in the wound should be removed by surgical debridement or alternative debridement methods, as they will prevent wound healing and pave the way for infection. These alternative methods include enzymatic, biological, otolytic and mechanical debridement techniques. (Guthrie, H. C et al;2011-24) (Nazarko L. Et al;2015-40) Deperiosteal bone and cartilage fragments that have lost their integrity in the wound, tendinous structures that have stripped the paratenon should be removed from the tissue because of their capacity to necrosis and prepare the ground for secondary infection. (Wysocki A. B. Et al;2002-65)

If there are microabscess foci that will cause localized abscess around the wound, they must be drained. If there is a cellulitis-like soft tissue infection in the surrounding soft tissues, appropriate local and systemic antibiotic therapy should be started.

In the presence of excessive purulent or exudative secretion at the wound site, wound irrigation should be performed at frequent intervals. Thus, the exudate amount should be tried to be reduced. The presence of secretion is a factor that impairs marginal epithelialization at the wound site. In addition, the presence of secretion is a nutrient medium for bacteria and increases the risk of secondary infection.

Each wound has its own characteristics. Accordingly, the type of dressing and its frequency should be determined. The type of dressing should be changed if necessary, and alternative dressing materials should be used, where the patient's wound may benefit more. Additional debridements should

be performed when necessary while the dressing process continues. (Lindholm, C et al;2016-35)

Finally, obtaining a wound site where there is no active infection, edema regressed, necrotic tissue and debris are cleared, secretion is minimized, and clean-looking live granulation tissue is observed will be evidence of the positive progress of the treatment process.

4. DRESSING TYPES AND MATERIALS

Developing technology and modernization have led to significant developments in the field of wound care. (Deng, X et al;2022-15) Wound treatment studies are becoming more effective with experimental animal models.

Many new products for use in wound treatment have been offered to physicians and patients, and new products are added to this list every day. (Oryan, A., et al;2016-43) Appropriate material and dressing should be selected as the treatment method according to the current characteristics of the wound. (Donelan S. Et al;2003-16)

Whichever product is chosen, the material used should be biocompatible, non-allergenic, non-toxic, cheap, easily accessible, easily applicable, not causing additional damage to the wound, and reliable. (Queen, D et al;2004-45) Dressing material should not adhere to the tissue to which it is applied, should allow drainage of exudate, provide painless application, have antibacterial properties, and allow tissue moistening. (Liang, Y et al;2022-34) (Vermeulen, H et al;2004-60) (Price, P. E et al;2008-44)

Wet dressing made with sterile gauze, which was used frequently in the past and is still preferred by most physicians today, is a dressing type that is easy to apply. They are passive effective covers. They only protect the wound surface. They do not affect wound healing. However, it causes loss of labor because it needs to be repeated at least 2-3 times a day to maintain the humidity of the environment, prevent adhesion, and prevent colonization. Considering the cost of other dressing materials and wound dressings, it is not difficult to understand why this dressing type is still often preferred. . (Zinat, N. J et al;2020-66)

Apart from wet dressing with physiological saline, topical antibiotic cream and pomade applications and the use of acid boric dry powder form are

quite common. In addition to the antimicrobial effects of the creams and pomades used, the humid environment and the preventive properties of the wound from drying provide an advantage. The acid boric powder form is used by sprinkling on moist gas or on the wound and its antipseudomonal activity is important. Apart from these, the silver dressing materials used show a rapid and effective antimicrobial effect due to the controlled release of ionic silver following exudate absorption. (Abedini, F et al;2013-1)

The choice of dressing should also consider the wound's size, depth, and location Apart from silver and wet dressings, creams, ointments, wound dressings are categorized as films, hydrogels, hydrocolloids, foams, alginates. (Roy, H et al;2020-53) Films and foams in polymeric structure are transparent-looking covers that allow gas passage and protect the humidity of the environment. (Walker, R. M et al;2017-61) Bioactive products called alginate, collagen, and hydrocolloids are in polymeric structure. (Held, M et al;2016-26) (El Masry, M. S et al;2019-19) They actively contribute to wound healing, thanks to their structural properties and the substances they release. (Alven, S et al;2022-3) Selection is made from these materials of different dressing types, which are suitable for the wound site, in other words, with appropriate biological properties according to the amount of exudation of the wound. (Fletcher J. Et al;2003-21) Hydrogel dressings are often preferred for moistening the dry wound surface. Alginates, on the other hand, are preferred for heavily exuding wounds due to their highly absorbent properties. (O'Meara, S et al;2015-42) the most important advantage of these materials is that they reduce the frequency of dressing application. They provide the advantage of changing frequencies from a minimum of 48 hours to a maximum of 1 week. In the selection of the material to be applied, it will be appropriate to choose the one that is suitable for the wound site. (Shi, C et al;2020-56) (SCALES J. T. Et al;1963-54) (Figure 1)

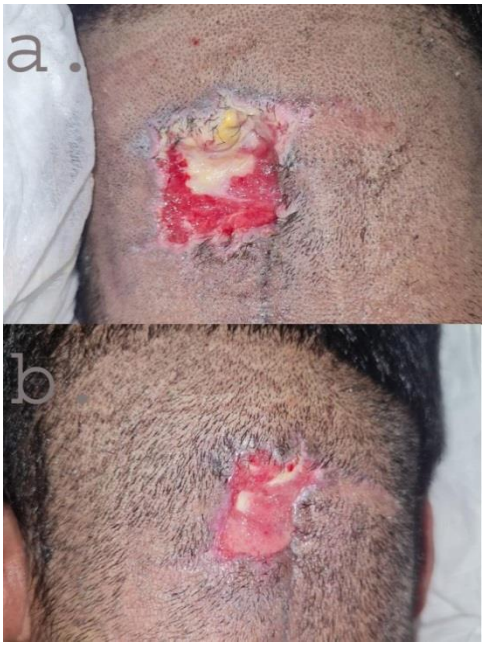

Figure 1. Before(a) and after(b) the patient using a collagen dressing

5. ALTERNATIVE TREATMENT METHODS

VAC (negative pressure wound treatment) is preferred especially for large and deep wounds, thanks to the negative pressure vacuum effect it creates. It is a closed-circuit dressing method that provides advantages in treatment due to its effective absorption of exudate, its contribution to wound contraction and its rapid effect on granulation tissue formation. (Agarwal, P et al;2019-2) It has advantages such as providing changes at intervals of 72 hours, as well as disadvantages such as preventing the patient's mobilization and lying position. (Figure 2)

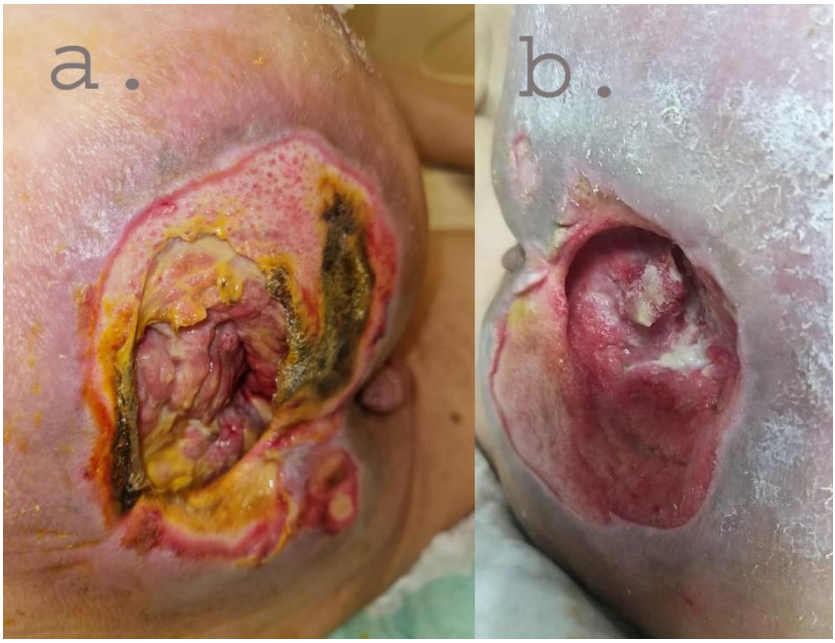

Figure 2. Sacral decubitus before(a) and after(b) VAC application

Ozone therapy, hyperbaric oxygen therapy, ultrasound and laser treatments, electrostimulation, maggot therapy, honey therapy, growth factors and stem cell therapy are among the other methods that can be preferred in wound care. (Messias de Lima, C. F et al;2017-38) (Moya-López, J et al;2020-39)

In hyperbaric oxygen treatment, patients are provided with 100% oxygen at 2-3 atmospheric pressure by using masks and headgear in special rooms. In order to avoid the toxic effects of high doses of oxygen, the treatment is done at least once a day in sessions not exceeding 2 hours for a period of several weeks. The positive effects of HBO treatment on angiogenesis, epithelialization increase, collagen synthesis increase, fibroblast cell proliferation and neutrophil functions have been proven by studies by increasing tissue oxygenation. . (Eisenbud D. E. Et al;2012-18) (Quirinia, A et al;1995-46)

Growth factors stimulate angiogenesis through cell proliferation, cell migration, cell differentiation, and increase in protein and enzyme synthesis. (Jimenez, P. A et al;1999-28), improvement in wound healing has been

achieved by taking advantage of the ability of these cells to transform into different cell types in stem cell applications. (Hassanshahi, A et al;2019-25) Platelet-rich plasma (prp) therapy has found use in wound treatment based on the fact that platelets are rich in growth factors. (Karayannopoulou, M et al;2015-29)

It is aimed to increase angiogenesis and collagen synthesis by creating stimulating effects on cellular mechanisms with electrical stimulation, ultrasound and laser applications, which have been used in recent years. (Chen, Y et al;2022-11) (Davis, S. C et al;1993-14) ozone therapy is another alternative treatment that can be applied topically or systemically. (Faraji, N et al;2021-20)

Honey application is a treatment method used in alternative medicine since ancient times. Its effectiveness has been proven in both experimental and human studies. The use of honey has taken its place in the modern treatment world because of keeping the wound area moist, facilitating epidermal migration, carrying antibacterial properties and containing trace elements effective in wound healing. (Iacopetti, I et al;2020-27) (Figure 3)

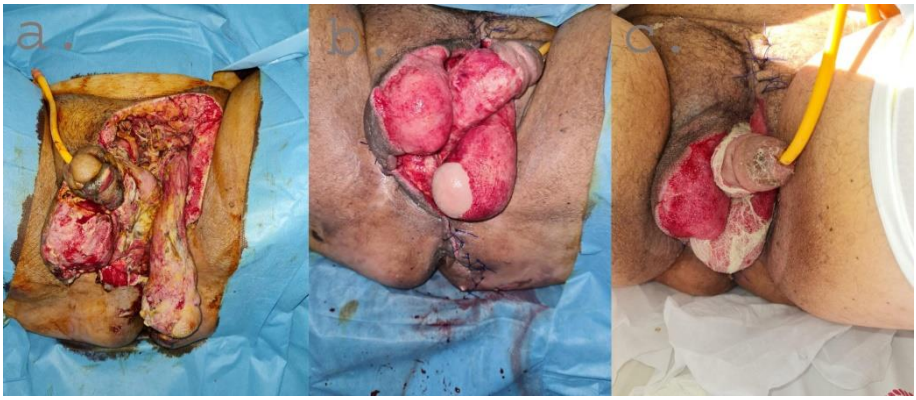

Figure 3. Fournier's gangrene after debridement(a) Results with VAC, collagenous dressing and topical honey application following debridement(b,c)

Thanks to rapidly developing technology, tissue engineering and genetic studies, products consisting of extracellular matrix components (integra) and acellular dermal matrix (alloderm) have begun to be used in wound care treatment. (Bondioli, E et al;2019-6) also found use of graft equivalent products called xenograft (from pig)

and allograft (from cadaver). In addition, studies are being carried out on new generation bioactive wound dressings that provide controlled release of active substances.

6. IMPORTANCE OF DEBRIDEMENT

Debridement is an application that aims to remove devital, necrotic, and infectious tissues in the wound site by surgical or alternative methods. (Attinger, C. E et al;2006-5) (Steed D. L. Et al;2004-57).

Wound debridement can be performed by surgical, biological, mechanical, autolytic and enzymatic methods. (Stephen-Haynes, J et al;2007-58) The most commonly preferred method is classical surgical debridement. With the help of surgical instruments and scalpel, it is aimed to remove the tissues that impair wound healing and to reach the bleeding tissue. Thus, it is aimed to accelerate the development of granulation tissue by vascular budding from bleeding foci. Serial debridement should be applied especially in problematic wounds during the wound healing process. In other words, targeted necrosis tissues at the wound site are removed from the wound bed in order to accelerate wound healing at certain day intervals. (Attinger, C. E et al;2001-4)

Mechanical debridement is the nomenclature that describes the removal of more fragile and fragile superficial necrosis and foreign bodies using physical force. The best example of this is cleaning by rubbing sterile gauze cloths on the wound. Another example of mechanical cleaning is pulsatile irrigation.

Enzymatic debridement is a type of debridement that aims to dissolve existing necrotic tissues by using topical substances with chemical properties instead of wounds. (Ramundo, J et al;2008-49) Biological debridement is a method popularly called maggot treatment. With this method, live sterile larvae are used to clean necrotic tissues. (Sherman, R. A et al;2017-55) Autolytic debridement describes the phagocytic process by the body's own cells. Here, the body's self-

healing capacity is utilized through daily changing dressings and topical agents. It is an easy but slow process. We often prefer silver creams for autolytic debridement. Recently, ultrasonic debridement devices using ultrasound energy have started to take place in the literature.

Among all these debridement methods, we should not forget that the most effective and fastest method is surgical debridement. Although it is more aggressive than other methods, we should not avoid using it for the sake of patients when necessary. Especially in severe clinical conditions such as necrotizing fasciitis and electrical burns, surgical methods such as escharotomy and fasciotomy are frequently performed before surgical debridement in cases where there is a risk of compartment syndrome and extremity circulation is compromised.

7. RECONSTRUCTION PYRAMID AND SURGICAL CLOSURE OF THE EXISTING DEFECT

Thanks to the debridement, dressing types used and wound dressings, after a certain period of time; problematic wounds are made into a place where we can perform the surgical procedure. The wound is basically considered to have reached the final stage when it is free from foreign bodies and necrosis, the edema regresses and the discharge is minimized, marginal epithelialization is observed, and viable bleeding granulation tissue is obtained as a result of neovascular tissue formation. Now the surgical team is at the stage of closing the existing open wound with the appropriate surgical method.

Wounds, in other words, defects are closed according to the classical reconstruction pyramid adopted by plastic surgeons. At the bottom of this pyramid are wounds that can heal spontaneously with primary suturing or dressings. As the severity of the wound and defect increases, skin grafts, local flaps, regional flaps, and in the most difficult cases, free tissue transfers are applied respectively when the appropriate ground is formed.

Traumatic injuries are mostly injuries that can be closed primarily if there is no contamination and foreign body presence, and if there is no obvious soft tissue defect, although it varies from region to region. If the existing defect will not leave a significant deformity in the anatomical location when primary closure, this should be preferred first. Careful surgical planning is required in areas such as the eyelid, ear, nose and mouth area.

Defect areas that are not suitable for primary closure should first be closed with skin grafts. (Kohlhauser, M et al;2021-31) If suitable granulation tissue is developed, preferably partial thickness or full thickness skin grafts can be applied according to the anatomical region to be applied. (Garcia, N et al;2023-23) In order to increase the adaptation rate of the skin graft, it is essential that there is no ongoing infection at the wound floor. Skin graft application should be postponed for a while if there is excessive secretion in the wound bed. The graft should be adapted so that it does not slide into the wound floor and no dead space should be left. Otherwise, hematoma and serous fluids that will accumulate under the graft will cause the loss of the graft. The presence of exposed bone, cartilage and tendinous structures at the wound base often presents an obstacle for skin grafting. In such cases, applications with better covering properties and vitality, such as flap tissue, should be preferred.

In cases where the existing wound is quite wide, the base is irregular, bumpy and has deep pouch characteristics, flap tissues should be preferred in surgery in order to cover the defective pit, which is a dead space, to cover structures such as bone-cartilage-tendon, and to obtain a more acceptable surface contour.

Flaps are grouped under local, regional and free flaps. Local flaps are flaps that can be designed in different ways, mostly showing a random circulation pattern, planned in the vicinity of the defect or wound. (Chang, J. W et al;2019-10) regional flaps are mostly flaps that have a feeding vessel of their own, have an axial circulation pattern, are not directly adjacent to the defect, but can be planned larger in size,

designed near the defect and transferred to the defect area in different ways over the feeder vessel. (Marcasciano, M et al;2017-36)

The flaps at the top of the reconstruction pyramid are called free flaps. These are the tissues that are freely taken from the donor area, determined based on microsurgical principles, together with its vein, and its circulation is restored by anastomosis to the recipient vascular structures in the planned area. (McCarty, J. L et al;2019-37) Thanks to this free transfer, functional muscle and nerve transfer, vital bone tissue transfer can be successfully applied. They are surgeries with a high level of difficulty and are performed in advanced centers where surgeons with extra training in microsurgery and appropriate equipment are available.

CONCLUSION

Wound treatment is an issue that can be achieved with a multidisciplinary approach. Under the leadership of plastic surgeons, physicians from the physical therapy, orthopedics, general surgery, neurology, nutrition and dietitian units organize appropriate treatment and support programs for patients. But most importantly, psychological support should not be missing for patients to get through this process. Protecting mental health is very important for people with such chronic diseases and wounds that disrupt their comfort of life.

The focus should be on which approach can be more effective for the patient and his wound, rather than which method is better in the treatment. Treatment can be applied with both old dressing methods and modern wound dressings. Because each dressing type and product has its own advantages and disadvantages. There is no product that can provide all ideal wound care conditions on its own. Seeing the fact that wound treatment is an important financial burden for the country's economy, it would be a smart approach to use the cheapest of the products that can be used and thought to be beneficial.

Experimental wound model studies that have been done and ongoing will help us to achieve more successful results in wound care treatment. (Dunn, L et al;2013-17) (Lee, J. H et al;2022-33) In order to

achieve success, we physicians must make patient and wound-based evaluations and not be prejudiced against any treatment method. It has been accepted by all wound care professionals that maintaining the moisture of the wound bed is the most important issue in the current approach.

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CHAPTER 5

CURRENT ADVANCES IN BREAST IMAGING

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INTRODUCTION

Breast cancer (BC) is the most common cancer in women with more than 2 million new cases annually and a high economic and physiological burden on both health care systems and the general population (WHO 2021; Taridu et al., 2022). BC is the second most common cause of mortality following cardiac disease (Wang 2018). Approximately 12% of women in European countries and the USA suffer from BC during their lifespan (Grosenick et al., 2016). Early detection of the disease is of paramount importance for the control and treatment. BC has a quite high survival rates when diagnosed early. There are currently three imaging modalities used in detection and screening for BC. These methods include mammography, MRI and ultrasound. These techniques are continuously refined and improved to improve sensitivity and specificity and early diagnosis of BC. With the advancement of breast imaging, today there are about 20 different imaging modalities that could help to improve diagnosis of BC. In this chapter, some of these novel approaches are addressed.

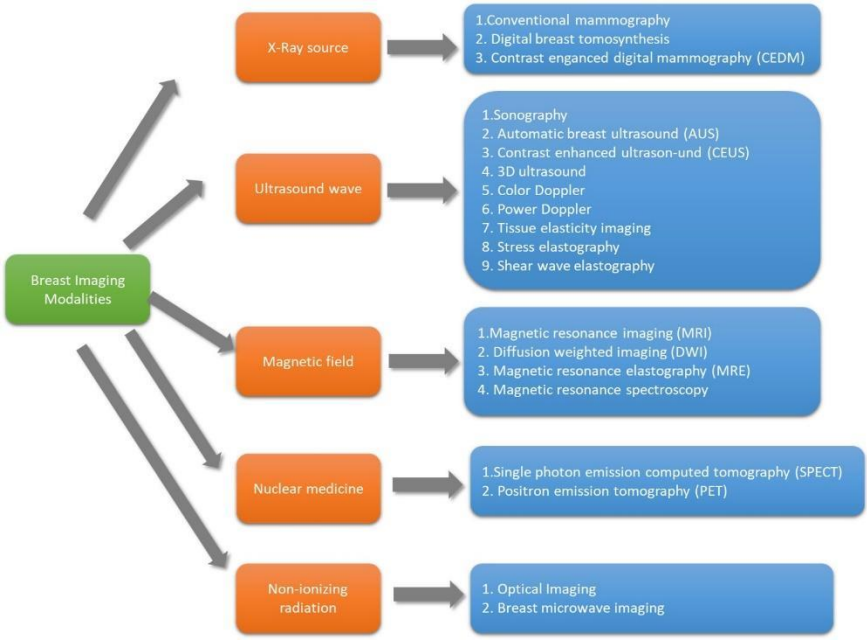
1. Breast Imaging

Breast imaging is one of the most critical subspecialties in the field of radiology. The importance of early diagnosis is highly recognized and the breast screening modalities are debated with several guidelines proposed all over the world (CDC 2022).

Today, the need for individualized medicine has changed our vision of this area, gaining more importance to multimodality and to integration of both artificial intelligence (AI) and human resources (HR). Increased mammographic breast density is a well established factor for the risk of developing breast cancer regardless of age (Gastounioti et al., 2021). The gold standard of categorizing breast density is the estimation of a radiologist based on American College of Radiology (ACR) Breast Imaging Reporting and Data System (BI-RADS) criteria (D'Orsi et al., 2019). The automated evaluation of breast density using the current softwares could be beneficial. In this method, breast cancers are depicted by artificial intelligence-based computer-assisted diagnosis (AI-CAD) (Tari et al., 2018).

The most recent advances in breast imaging include digital breast tomosynthesis (DBT), automated breast ultrasound (ABUS) and contrast-enhanced spectral mammography (CESM). In addition, the assessment of ultrafast MRI could have a major role in BC prevention, especially in women

at risk. In this section, the most recent advances in breast imaging is discussed briefly in the light of the current literature (Figure 1).



1.1. Multiparametric Breast MRI

Technical advancements have improved the quality of breast MRI, enabling the acquisition of isotropic high-resolution images. Multiparametric breast MRI has largely replaced the conventional approach, which was mainly based on conventional contrast-enhanced sequences alone for the classification of lesions (Mann et al., 2019). MRI performed by experienced teams improves surgical practice, reducing re-excisions and prevents unnecessary mastectomies. MRI allows selection of patients for neoadjuvant chemotherapy. It is the modality of choice for modifying therapeutic agents, for preoperative assessment of residual tumor size in order to determine the candidates for breast conserving surgery. From an economic viewpoint, breast MRI can be optimized by adjusting the protocol based on indications.

1.2. Diffusion-weighted Imaging

Diffusion-weighted imaging (DWI) is utilized in order to improve the specificity of MRI, and to provide physiological data about the movement of water in abnormal versus normal breast tissue and functional environment.

Quantitative study of apparent diffusion coefficient has been demonstrated to be beneficial in distinguishing benign from malignant masses, because malignant lesions typically exhibit restricted diffusion of water. Recent studies have reported that glandular tissue normalized apparent diffusion coefficient value further improves the performance of DWI. This improvement exceeds the performance obtained with conventional 3D T1-weighted and dynamic MRI (Ei Khouli et al., 2010).

1.3. Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is a novel non-invasive modality that can improve the specificity of dynamic contrast-enhancing MRI. MRS of the breast region assess for a 3.2 parts per million resonance peak, which indicates an increase in the levels of choline metabolites (Melsaether and Gudi, 2014).

Total choline is a biomarker of increased turnover of cell membrane and although it is mostly detected in malignant lesions, because malignant lesions typically exhibit restricted diffusion of water (Melsaether and Gudi, 2014). In a meta-analysis conducted by Baltzer and Dietzel including 19 MRS breast analyses, promising results have been reported with a sensitivity of 73% and specificity of 88% (Baltzer and Dietzel, 2013). MRS modality has also been evaluated in response to neoadjuvant chemotherapy and yielded different results (Melsaether and Gudi, 2014).

1.4. Digital Breast Tomosynthesis (DBT)

Digital Breast Tomosynthesis (DBT) is a sub-modality of mammography. Its difference is the rotating X-ray tube around a narrow angle of 15-60 degrees from the tissue, which is compressed and thus, providing 3F information about the breast (Mortezazadeh et al., 2020). DBT images are produced through repeated exposure of breast tissue from different angles and are reconstructed as half mm thickness slices. Studies have indicated that this modality increased the dose of radiation by 20%, but improved the rate of cancer detection by 20% and decreased recall rate by 15 to 30% (Gilbert and Pinker-Domenig, 2022). An advantage of this modality is the identification of lesions that may not be observed in mammography due to overlapping with dense tissue of the breast. The high sensitivity and low rate of false positives in DBT contribute to more accurate breast cancer staging. Major limitation of this modality is low sensitivity in detection of microcalcifications (Drukteinis

et al., 2013). DBT increases the diagnosis of breast cancer by 27% and decreases false-positive findings by 15% (Greenwood et al., 2018). In addition, DBT reduces recall for other medical investigations.

1.5. Contrast enhanced digital mammography (CEDM)

CEDM is a promising method that uses iodinated contrast to detect tumor vascularity. Several studies have reported that the microenvironment in the tumor is associated with metastasis (Dromain and Balleyguier, 2010). CEDM is the second type of advanced technology originating from the digital platform. CEDM is based on the success of breast MRI, which has currently the highest sensitivity rate of 98% (Morris et al., 2003). CEDM is an alternative technique at combining contrast enhancement with mammography. In this method, 1.5 mL/Kg nonionic iodinated contrast agent is used. CEDM can be performed using a standard mammography unit by addition a copper filter and software upgrades to make the equipment capable of dual energy imaging (Sogani et al., 2021). The standard digital mammography unit was modified to enable the use of a specialized filter that can shape the X-ray spectrum to perform CEDM (Jochelson and Lobbes, 2021). CEDM provides similar physiologic and morphologic information similar to contrast enhanced breast MRI.

1.6. Automatic Breast Ultrasound

The automatic breast ultrasound (ABUS) is a computer-based system for assessment of the whole breast. ABUS was developed in order to obtain an operator independent system. It has the advantage of a high diagnostic accuracy. In addition, it better determines the lesion size (Mortezazadeh et al., 2020). Three positions are obtained by the operator from each breast including anteroposterior, medial and lateral. For optimal image quality, additional superior and inferior views are taken to avoid the exclusion of upper and deep breast tissue. Then the images are reconstructed in 3D. This method is better in women with dense breasts. ABUS has been shown to reduce the recall rate by less than 2.5% (Gilbert and Pinker-Domenig, 2022). ABUS has a 79% sensitivity and 83.3% specificity in the diagnosis of breast cancer (Guo et al., 2018). Today there are two types of ABUS as prone- and supine-scanners (Kaplan, 2014). The ABUS scan is continuous and automated. Volume acquisitions are made in the axial plane starting from the inferior part of the breast with sagittal and coronal reconstruction. ABUS is reported as a comfortable and time-efficient method (Vourtsis and Kachulis, 2018).

1.7. Contrast Enhanced Ultrasound (CEUS)

Tumor vascularization is based on its severity and size. CEUS isan imaging modality used in clinical studies to monitor different vascular structures. CEUS in breast pathology has a wide area of usage including differentiating benign from malignant lesions, and evaluating the extent of the disease in order to evaluate the efficacy of neoadjuvant therapy (Nykänen et al., 2017). In order to improve the return of the waves, gas microbubbles between 1-7 μm are injected in CEUS technique. Since these bubbles are more echogenic compared to red blood cells, they can not pass the vascular walls. The ultrasound waves destroy the bubbles and the gas is eliminated through the lungs. There are differences in impedance, compression and acoustic features between the microbubbles and surrounding tissues (Guo et al., 2018). CEUS has been found to improve diagnostic efficacy compared to unenhanced ultrasound and mammography (Shao et al., 2020). CEUS provides information about blood perfusion from tumor-induced vascularity (Zhou et al., 2020). Studies have demonstrated that CEUS increases accuracy in distinguishing malignant from benign breast lesions (Yuan et al., 2013). In CEUS, the patient is examined in supine position and the procedure lasts about 15 minutes including preparations. CEUS also seems to be a promising technique to monitor response during and after neoadjuvant therapy (Boca et al., 2021; Lee et al., 2019). This method is an ongoing field of research and is not yet recommended for clinical use.

1.8. Three-dimensional Ultrasound

The introduction of 3D ultrasound enables acquiring three dimensional breast lesions, facilitating estimates of the lesion volume, which is complicated with 2D ultrasound (Cho et al., 2005). 3D ultrasound has two main forms. In the first form, 2D imaging equipment is used with special mechanics and reconstructs of 3D volume are then reconstructed. In the second form, 3D volume is scanned electronically using a matrix array converter (Guo et al., 2018). A beam is created by the matrix converter in both positions and a pyramidal volume is formed. 3D ultrasound provides the comprehensive information of all 2D lesions and as well as offers information of the coronal plane simultaneously. These images are then elaborated to highlight ultrasonographic characteristics of the lesion and provide new diagnostically useful information (Abbattista et al., 2007).

1.9. Color Doppler

The color Doppler provides information about the local flow of the blood through mean Doppler shifting code in the region of interest (ROI) and color this region. It is possible in color Doppler to choose the average frequency shift as a variable to show blood flow. The signals of Doppler are generally increased in malignant breast lesions. This technique highly depends on technical factors. The sensitivity of color Doppler increases with the reduction of color box size. Reducing pressure of the transducer on the breast also affects color Doppler. Relatively superficial breast lesions may cause vessels to easily close when squeezed between the chest wall and the transducer (Carpentier et al., 2017).

Color Doppler is useful in differentiating cystic lesions from solid lesions, distinguishing malignant from benign lesions, determining aggressiveness of suspicious tumors, evaluating inflammatory conditions of the breast, and assessing response to tumor therapy. Color Doppler can show an internal vessel, indicating that a lesion is solid. It demonstrates the presence of blood vessels within pseudocyctis lesions (Kwak et al., 2008). Inıtial studies conducted using color Doppler have reported that breast tumors as small as 10 mm are associated with abnormal flow patterns (del Cura et al., 2005). Color Doppler has been shown to assign a high level of suspicion to a lesion for being malignant. However, the definitive role of color Doppler sonography is yet to be established.

1.10. Power Doppler

Power Doppler is a method encoding pulse power into the Doppler signal and shows the signal in a single color. Since the frequency is identified by the velocity of red blood cells (RBC), strength of the signal depends on the amount of blood in the target region. The direction of the flow is often not important. Power Doppler has several advantages such as better resolution in the edges and high sensitivity to blood flow. Power Doppler is in general used for the diagnosis of solid breast masses (Carpentier et al., 2017). Power Doppler allows demonstration of slow flow pattern without taking into account directional data. Power Doppler can show neovascularization and arteriovenous shunts in neoplastic lesions to distinguish malignant from benign lesions (Huang et al., 2009). It has been reported that the vascular indices obtained with power Doppler seem reliable in the further

characterization of suspicious masses and might be used to decrease unnecessary biopsies (Kupeli et al., 2016).

1.11. Tissue Elasticity Imaging (Sonoelastography)

The use of ultrasound for imaging tumor elasticity has been recently introduced in order to improve sensitivity and specificity of ultrasound in the diagnosis of breast cancer. Solid tumors are stiffer than the surrounding tissues. The stiffness of a tissue is determined using elasticity, also known as Young's. The Young modulus of the soft tissue ranges from 1 to 100 kPa, while elasticity of breast tumor is 15 times stiffer compared to the soft tissue. Before and after compression, sonograms are produced in this procedure. Deformation of the solid lesions is less and thus, these lesions appear darker in an elastogram. Malignant lesions appear darker than benign ones. Whereas, benign lesions can be darker or lighter than the surroundings. The breast lesions' sizes are compared between B-mode and elastogram to reveal differences between malignant and benign masses. Fibroadenomas and cysts have the same size in B-mode and elastogram, while carcinoma appears larger in elastography (Mortezazadeh et al., 2020). Specificity of sonoelastography is higher than that of B-mode. Sonoelastography has a 88.5% sensitivity and 92.7% specificity.

Sonoelastography technique is generally performed using stress elastography (SE) shear wave elastography (SWE) (Barr, 2018). Sensitivity and specificity of these two forms are similar.

1.11.1. Stress Elastography (SE)

Stress elastography, which estimates tissue stress, is an adjunct to conventional ultrasound. Stress elastography examines the change in texture under an external force. SE measures axial displacement of tissue caused by mechanical stress in real-time (Carlsen et al., 2013). In the face of an ultrasound wave, soft tissue changes more compared to stiff tissue. This tissue is subjected to an external force and the desired image is acquired under these conditions and combined with non-force stage imaging. In stress elastography, a complex software is not needed, but this method depends highly on the user performance (Barr, 2018; Kim et al., 2018).

1.11.2. Shear Wave Elastography (SWE)

Ultrasonic pressure in the material results in volume changes that leads to the production of transverse ultrasound waves known as shear waves. Shear waves move faster in stiff tissues compared to soft tissues.

Wave velocity is between 1-50 m/s, while frequency is between 10-2000 Hz. Shear wave elasticity is expressed as Young's module in kPa or m/s. In SWE, ultrasound itself applies a dynamic compression in the ROI and elasticity of the wave is then converted into an image. When a ROI of is chosen, pre-compression is removed and 2D SWE is turned on. The patient must stay motionless during the acquisition (Kim et al., 2018).

1.12. Nuclear Medicine Imaging

In the field of oncology, nuclear medicine is used for diagnosis, planning and evaluating response to treatment applied. In this technique, several specific radiopharmaceuticals are injected intravenously and images that show uptake of the drug by organs are collected (Mortezazadeh et al., 2020). In general, nuclear imaging of the breast is performed by positron emission tomography of breast (PET) and single-photon emission computed tomography (SPECT). Whereas SPECT is rather a physiological imaging modality, PET is a molecular and metabolic imaging technique (Groheux et al., 2016). The sensitivity of SPECT is low for small lesions because of the lower spatial resolution. In addition, the dose of radiation is about 20 to 30 folds higher in SPECT compared to digital mammography (Drukteinis et al., 2013). SPECT has a sensitivity of 96.4% and specificity of 59.5%. Low specificity as well as high radiation dose has limited the use of SPECT in low-risk patients (Brem et al., 2009).

Positron emission tomography (PET) is a modality used to quantify cells' physiological and biochemical activities at molecular levels (Groheux et al., 2016). PET is often used to identify the grade, stage, and to evaluate response to treatment. Increased uptake of glucose in malignant cells has been known for a long time. Therefore, one of the most widely used PET tracers is ^{18}F-2-deoxy-D-glucose, which is the fluorinated analog of glucose. PET has a higher spatial resolution compared to SPECT. Therefore it can detect smaller lesions. PET has a 90% sensitivity and 86% specificity (Marino et al., 2015). PET is an expensive method with 3.7 times higher cost compared to SPECT. For these reasons, SPECT and PET are not commonly used in low-risk cases.

CONCLUSION

Breast imaging is performed primarily with three techniques including mammography, ultrasound and MRI. All three methods are undergoing continuous attempts to increase their sensitivity and specificity especially for distinguishing malignant from benign lesions, determining aggressiveness of a tumor, staging and eliminating unnecessary biopsies. Today, there have been numerous modifications, improvements and refinements in these methods to obtain better outcomes. It is important for clinicians to pursue these attempts and follow new advances in breast imaging. Integration of artificial intelligence (AI) and machine learning (ML) to these modalities seems to result in significant changes and shifting paradigms in the diagnosis and treatment of breast cancers.

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CHAPTER 6

TRANSIENT TACHYPNEA OF NEWBORN

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INTRODUCTION

Transient tachypnea of newborn (TTN) is a parenchymal pulmonary disorder characterized by pulmonary edema due to delayed resorption and clearance of fetal alveolar fluid. TTN is a respiratory problem, which can be encountered in the newborn shortly after birth. TTN is the most common cause of respiratory distress in newborns. In a review of 33,289 term deliveries, the incidence of TTN was 5.7 per 1,000 deliveries. TTN consists of a rapid breathing. It is likely a result of retained lung fluid and is mostly seen in infants of >35 gestational age who are delivered by cesarean section. It is often self-limiting and usually resolves within 24 to 72 hours of life.

Risk factors for developing TTN include male gender, lower gestational age, elective cesarean section, maternal diabetes and maternal asthma. Clinical presentation include respiratory distress in late-preterm or term infants withn the first hours of life, tachypnea, retraction, grunting, nasal flaring, cyanosis and barrel chest in some newborns. Investigations in TTN include pulse-oxymetry monitoring, blood gas and glucose level, chest X-ray, septic examination and ECG in the case of suspicion of congenital heart disease. The diagnosis of TTN is established if the newborn is systematically monitored, if requires FiO2 >40%, if needs ventilation and when the signs persists for more than 72 hours.

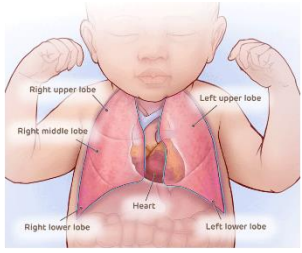
This chapter begins with the definition of TTN. Epidemiology, risk factors, diagnosis, management, prognosis and prevention of TTN are discussed in detail in the light of the current literature.

1. DEFINITION

Transient tachypnea of the newborn (TTN), also known as “Wet Lung”, is a self-limiting disease characterized by pneumonia caused by delayed resorption and clearance of detal alveolar fluid that lead to insufficient gas exchange. In the immediate newborn period, namely term or near-term, TTN is a common cause of respiratory disorder (Bruschettini, Hassan and Romantsik et al., 2022). TTN is a term used for a mild respiratory problem of newborns, which begins after delivery and last for about three days. Transient tachypnea is characterized by very fast breathing rate due to too much fluid in the lungs of newborns. This fluid limits the amount of oxygen pulled into the lungs of newborns, resulting in a faster breathing. The

infants born with this condition usually recover within 72 hours wşthout the need for medical intervention.

Several steps that must occur for the lungs to allow for adequate oxygenation and ventilation are shown in Figure 1 (Guglani, Lakshminrusimha, Ryan et al, 2008).



- ❑ Establishment of continuous breathing
- ❑ Alveoral distension
- ❑ Clearance of lung fluid
- ❑ Secretion of surfactant
- ❑ Decreased pulmonary vascular resistance and increased pulmonary blood flow
- ❑ Cessation of the right to left shunting followed by closure of ductus arteriosus

Figure 1. Steps of ventilation and oxygenation of fetal lungs

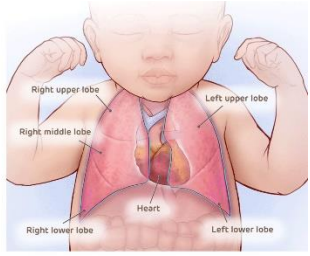
TTN is the most commonly encountered etiology of respiratory distress in newborns at term admitted to a neonatal intensive care unit (NICU). It is characterized by the early onset of tachypnea that is relieved by minimal ocygen supplementation (<40%) (Liem, Huq and Ekuma et al., 2011). TTN may lead to an increase in monitoring of the newborn, separation from the mother, the need for respiratory support and several interventions including antibiotics and prolonged hospitalization (Lakshminrusimha and Keszler, 2015). The symptoms of TTN overlap with a wide range of pathophysiologic states. There is always a dilemma in the diagnosis of TTN because of the lower sensitivity and specificity of clinical signs and symptoms (Elemraid, Muller and Spencer, 2014).

2. EPIDEMIOLOGY AND RISK FACTORS

TTN was described for the first time in 1966 as a clinical presentation of delayed clearance of fetal lung fluid (Avery, Gatewood and Brumley, 1966). Studies on the prevalence of TTN are limited in the literature. However, current studies have reported the prevalence of TTN as 4.0-5.7 per 1000 term deliveries (Ryan and Huges, 1995; Alhassen, Vali and Guglani,

2021). Despite TTN being one of the most common causes of neonatal RDS, many TTN cases may be underdiagnosed (Greenough, 2006). The estimated incidence of TTN has been reported between 0.5% and 2.8% of all deliveries as a result of delayed clearance of lung fluid (Agarwal, Dabid and Harris, 2003). In most cases, TTN occurs in late preterm or term newborns between 37-39 gestational weeks (Derbent, Tatli, Duran et al., 2011). It has been reported in a study that TTN in about 10% of newborns with 33-34 gestational weeks, 5% of newborns with 35-36 gestational weeks and less than 1% in term infants (Raju, Higgins, Stark et al., 2006).

Risk factors for the development of TTN include urban birth location, male gender, low Apgar scores and birth weight ≥4500 g. In addition, TTN-diagnosed newborns are at a higher risk of developing a wheezing disorder in childhood (Liem, Huq, and Ekuma et al., 2007; Kahvecioğlu, Çakır and Yıldız, et al., 2016). In addition, delivery before completion of 39 weeks gestation, multiple gestations, a cesarean section without labor, maternal history of asthma, hypertension and gestational diabetes have been associated with an increased risk for development of TTN (Badran, Abdalgani and Al-Lawama et al., 2012;). Other obstetric factors such as excessive maternal sedation, volume of maternal intravenous fluid and prolonged labour have been less implicated (Hermansen and Lorah, 2007). Risk factors of developing TTN are shown in Figure 2.



- ❑ Delayed cord clamping
- ❑ Rapid vaginal delivery
- ❑ Excess maternal fluid administration
- ❑ Male gender
- ❑ Preemies and full-term infants
- ❑ Delivered by caesarean section
- ❑ Born to mothers with diabetes
- ❑ Born to mothers with asthma
- ❑ Small for gestational age

Figure 2. Risk factors for developing TTN

3. PATHOPHYSIOLOGY

In the past, it was believed that respiratory distress is a problem of relative surfactant deficiency, but is now characterized by inability to absorb fetal lung fluid. In the fetal fluid filled lung, the fluid is actively secreted by the pulmonary epithelium. The fluid is produced as 2 mL/Kg/h in the beginning of pregnancy and increases to 5 mL/Kg/h at full term. The onset of lanour increases the level of epinephrine in the circulation, which is thought to activate the switch from secretion to reabsorption in the lungs (Brown, Olver and Ramsden et al., 1983). The fluid within the lungs remains constant at 90–95% of total lung weight through much of the third trimester, but begins to reduce a few days before spontaneous vaginal delivery. The inability of the immature fetal lung to switch from fluid to absorption mainly because of immaturity in expression of the epithelial Na+ channel (EnaC), causes development of hypoxemia, and fetal lung fluid retention. TTN develops in mature newborns with poorly developed respiratory Na^+ transport (Bricelj, Tul and Lucovnik et al., 2017).

According to another possible mechanism, during a vaginal birth and particularly with full-term infants, the pressure of passing through the birth canal squeezes some of the fluid of the newborn. In addition, physical pressure during labour and hormonal changes may also lead to push out some of the fetal fluid (Bruschettini, Hassan and Romantsik et al., 2022). Premature infants or those who are delivered by a rapid vaginal delivery or cesarean section do not undergo usual hormonal change and squeezing of a normal vaginal delivery. Therefore, these newborns tend to have more fluids than normal in their lungs, during first breaths. This excess fluid prevent some oxygen to move from lungs to the blood, leading to the development of TTN (Aathi, 2014).

4. CLINICAL PRESENTATION AND DIAGNOSIS

TTN is a diagnosis of exclusion and can not be established unless other causes of respiratory distress are ruled out.

4.1. History

The maternal history in TTN consists of cesarean section without labour and precipitous delivery. In a short time following birth, signs of

respiratory distress become evident. These signs include tachypnea, retractions, increased need for oxygen, nasal flaring, grunting and hypoxia.

4.2. Physical Examination

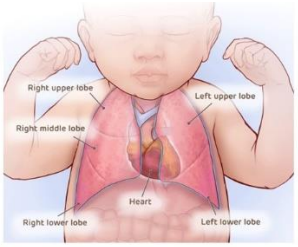
Physical findings in newborns with TTN include tachypnea with retractions, grunting and flaring. Extreme cases may show severe hypoxia and cyanosis. Typically the tachypnea is between 60-80 breaths per minute; however, can sometimes be substantial. It was reported in a study that peak respiratory rate of >90 breaths per minute during the first 36 hours of life was associated with prolonged tachypnea lasting more than 72 hours (Kasap, Duman and Ozer et al., 2008).

4.3. Other Diagnostic Methods

Chest X-ray of a newborn with TTN appears streaked and the fluid may be seen. Chest X-ray can show retained lung fluid as diffuse streaky pulmonary interstitial opacities and fluid in the fissures. Ultrasound of the lung has become a widely used method in the diagnosis of TTN (Ibrahim, Omran, and AbdAllah et al., 2018). Experienced neonatal lung ultrasonographers can distinguish between RDS and TTN.

Pulse-oximetry monitoring provides information on how well the lungs are sending oxygen to the blood. This monitoring is also useful in monitoring TTN. A complete blood count may also be performed from a vein or heel of the infant to control signs of infection.

Respiratory stress is not solely TTN and not a more serious disorder such as sepsis, pneumonia, cyanotic congenital heart disease and persistent pulmonary hypertension. This limits the evaluation of the newborn and in this case there is a potential for misdiagnosis and inappropriate treatment. Differential diagnosis of TTN is shown in Figure 3.



- ❑ Cerebral Hyperventilation
- ❑ Congenital Heart Disease
- ❑ Congenital Pneumonia
- ❑ Secretion of surfactant
- ❑ Meconium Aspiration Syndrome
- ❑ Metabolic Acidosis
- ❑ Neonatal Sepsis
- ❑ Persistent Pulmonary Hypertension of the Newborn
- ❑ Pneumomediastinum
- ❑ Pneumothorax Imaging
- ❑ Respiratory Distress Syndrome

Figure 3. Differential diagnosis of TTN

5. MANAGEMENT

Since the risk factors for developing TTN, including C/S, maternal diabetes and familial history of asthma are widespread, most newborns are at risk of developing TTN. Immediately after birth, grunting, which is considered a part of transition can be common. In a cohort study with 466 newborns, grunting was observed in 17.4% of the newborns, but the majority of gruntings resolved within 2 hours of life (Yost, Young and Buch, 2001). If the signs of respiratory distress persists, the infant may require further evaluation and intervention. Considering the limited resources, newborns with TTN often require transfer to a higher level of care, which separates the newborn from the mother. According to the Rule of 2 hours (also known as Heins' rule) ; two hours after onset of the respiratory stress, if the condition of an infant has not improved or worsened, if FiO2 more than 0.4 is required or if a chest X-ray is needed, the newborn should be transferred to a center with a higher level of neonatal care (Hein, Ely and Lofgren, 1998).

Routine care in NICU include continuous cardiopulmonary monitoring, maintaining neutral thermal environment, providing IV access, blood gas evaluation and evaluation for sepsis.

5.1. Respiratory management

If pulse-oximetry or arterial blood gas suggests hypoxia, oxygen support may be required. The preferred initial method is an oxygen hood, but nasal cannula or CPAP can also be considered. The concentration should be adjusted to an oxygen saturation in a low 90s level. In a study by Osman et al. in the infant group that received CPAP the duration of tachypnea was shorter and treatment resulted in less admissions to the NICU (Osman, El-Farrash and Mohammed, 2019). Requirement of ECMO or endotracheal intubation is in general uncommon; however, these should be taken into account in newborns with worsening respiratory status. Considering that there is a higher respiratory distress in late preterm and term infant delivered by elective C/S, the number of newborn with TTN who undergo ECMO may become a concerning trend, but overall need for ECMO in infants with TTN is very low (Guglani, Lakshminrusimha, Ryan et al, 2008) Arterial blood gas analysis should be repeated and pulse-oxymetry monitoring should be continued until the signs of TTN are resolved (Jha, Nassar, Makker, 2022).

5.2. Nutritional Management

The degree of nutritional support needed is usually determined by the newborn's respiratory status. Oral feeding is usually unsafe in the case of tachypnea of over 80 breaths for minute associated with increasing work of breathing (WOB). Supportive nutritional management includes intravenous fluids (IV) and gavage feedings until the respiratory rate has decreased enough to allow oral feedings. IV fluids should be started at 60-80 mL per Kg per day in these infants. Enteral feeding can be initiated if respiratory distress resolves, diagnosis is definite and respiratory rate is less than 80 breaths for minute. Enteral feeding should be started slowly with progressive increments until TTN is resolved (Jha et al., 2022).

5.3. Infection Management

If infection is suspected because of clinical symptoms and risk factors, a complete blood count, C-reactive protein and blood culture should be performed. Empiric therapy with antibiotics such as ampicillin and gentamicin should always be considered, because TTN may be difficult to distinguish from neonatal sepsis and pneumonia. However, routine use of empiric antibiotics in TTN may be detrimental, leading to alterations in gut microflora and an increase in antibiotic resistant organisms (Cotten, 2016).

However, since it is challenging to rule out sepsis and pneumonia clinically, especially in the absence of risk factors, empiric antibiotics are used 48 hours after birth, until sepsis has been ruled out.

5.4. Medical Treatment

The use of medications in transient TTN is minimal. The efficacy of furosemide (Kassab, Khriesat, and Anabreesor, 2015) and racemic epinephrine (Kao, Stewart, and Belfort, 2008) was investigated in randomized controlled studies and it was found no significant difference in terms of the duration of TTN and length of stay in hospital compared to the control subjects. It has been shown that salbutamol decreased the duration of TTN symptoms and hospitalization, but further studies are needed to confirm its safety and efficacy (Armangil, Yurdakök and Korkmaz et al., 2011; Kim, Yoo and Jung et al., 2014). Central lines may be inserted if the newborn is mechanically ventilated, has an oxygen >40% or is hypotensive and requires vasoactive medications.

6. PROGNOSIS

The newborns with TTN usually recover fully after these infants receive special monitoring and treatment in hospital. Even after TTN resolves, signs and symptoms of respiratory stress should be monitored and neonatalogists should be consulted when suspecting a problem. If the infant appears blue, has trouble breathing, or if the skin pulls in between the ribs a neonatalogist and energency services should be aclled at once. This condition usually resolves within 24-48 hours of life after birth. Newborns with TTN usually have no further problems from this condition, and do not require special care or follow-up. In some case reports, malignant TTN has been reported in which persistent pulmonary hypertension has occurred due to elevation of pulmonary vascular resistance due to retained lung fluid (Lakshminrusimha and Keszler, 2015).

7. PREVENTION

Since the exact cause is unknown, there is no guidelines for prevention of TTN. There are several factors to consider for delivering a healthy infant. These factors include eating a healthy diet, aiming low in saturated fats and eich in fruits, vegetables and grains; regular prenatal check-ups, not smoking and avoiding drugs and alcohol.

CONCLUSION

Transient tachpynea of newborn is the most important respiratory problem in the neonatal period and is usualy resolves without coplications. Risk factors for TTN include male gender, small for gestational age, multiparity, maternal diabetes, maternal asthma and delivery by cesarean section. TTN requires increased monitoring, transfer to NICUs and delayed discharge. Other important diagnoses should be taken into account in the differential diagnosis. The overall prognosis for TTN is usually good. However, the pathophysiology of lung fluid clearance, which causes TTN, remaisn an important area of research in the field of neonatalogy to improve our understanding of TTN.

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CHAPTER 7

HUMAN PAPILLOMAVIRUS (HPV) AND GENOME EDITING TOOLS TO TARGET HPV E6 AND E7 ONCOGENES

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INTRODUCTION

Cervical cancer is fourth most prominent cancer in women and the eighth most frequently diagnosed cancer among all cancers worldwide with around 570,000 new cases and 310,000 deaths reported in 2018 (Bray et al., 2018). In Turkey, each year around 2400 women are diagnosed with cervical cancer and nearly 1300 die from the cancer. Cervical cancer-related deaths mostly occur in low-income disadvantaged countries (LaVigne, Triedman, Randall, Trimble, & Viswanathan, 2017). This is probably due to devoid of screening and immunisation programmes, and public awareness, unavailability of proper diagnostic tests, and treatment protocols as well as higher risk factor exposure that leads to cervical cancer. While the major causative factor is Human Papillomavirus (HPV) infection (zur Hausen, 2002), others include smoking, continuing utilisation of contraceptives, immune system suppression, multiple amount of childbirth (AmericanCancerSociety, 2021). Epidemiological studies indicate that the viral infection rate is almost 80% among sexually active women (Chesson, Dunne, Hariri, & Markowitz, 2014). However, in most cases viral infection is cleared by host immune system. Given that only persistent infections may result in cervical cancer (Winer et al., 2011), it can be said that it a rare condition triggered by ordinary HPV infections (Woodman, Collins, & Young, 2007). The most common communicable agents leading to cancer are *Helicobacter pylori* (36.3%) and HPV (31.1%), with hepatitis B (16.3%) and C (7.1%) (Plummer et al., 2016) coming after.

Although persistent HPV infection cannot be effectively treated, HPV-related cervical cancer is vaccine-preventable and the repeated screening programmes allow early detection and prevention of infection and cervical lesions (AmericanCancerSociety, 2021). Although the availability of an effective HPV vaccine, adding it to the national immunisation programmes can be problematic especially in developing countries due to its high cost (Levin et al., 2015; Pogoda, Roden, & Garcea, 2016). Also, in overpopulated countries, the commercially available multivalent HPV vaccine cannot cover all the major high-risk HPV types (Zhai & Tumban, 2016). In addition, as being a prophylactic vaccine, it will not be effective in people who are already infected with the virus. Therefore, alternative therapeutic strategies in treatment of HPV infection and related cervical cancers still need to be emerged. HPV infection can be targeted by using several therapeutic approaches. Here, HPV genome, its major oncoproteins E6 and E7, key

elements of tumorigenesis, hallmarks of cancer and employing genome editing for the disruption of E6/E7 oncogene, leading to elimination of HPV infection will be discussed in detail.

HPV AND CERVICAL CANCER

Human papillomaviruses are small, (with 50-55 nm in diameter) double stranded, round and non-enveloped DNA viruses that belong to the family of Papillomaviridae (Doorbar, Egawa, Griffin, Kranjec, & Murakami, 2015). Nucleotide sequence alignment studies of the L1 ORF displayed 222 different papillomavirus types, that showed less than 90% similarity, which then be allocated into genera, species, types, and subtypes (de Villiers, Fauquet, Broker, Bernard, & zur Hausen, 2004). Human papillomaviruses include five genera: Alpha (HPV16, 18, 31, 33, 52, etc. 65 different types), beta (HPV5, 8, 9, 12, 47, etc. 54 different types), gamma (HPV4, 48, 60, 65 etc. 100 different types), mu (HPV1, HPV63 and HPV 204), nu (only HPV41) (www.hpvcenter.se/human_reference_clones/accessed on January 20, 2023). These viruses are subdivided into cutaneous if they infect epithelial skin cells or mucosal types if they infect the cells from inner tissues. Contribution to cervical carcinogenesis by various mucosal or cutaneous HPV types differs and according to the potential oncogenicity these viruses are classified into three groups: high-risk, possibly high-risk, and low-risk HPV types. High-risk HPV types consist of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 (IARC Group 1-mucosal alpha types) & 68 (IARC Group 2A), possibly high-risk ones include HPV 5, 26, 53, 66, 67, 68, 70, 73, 82, 30, 34, 69, 85 and 97 (IARC Group 2B), and low-risk ones include HPV 6, 11, 42, 44, etc. (IARC Group 3) (Bouvard et al., 2009). Amid the high-risk HPVs, HPV16 accounts for 61.7% of the squamous cell carcinoma of cervix and 50% of adenocarcinoma of cervix worldwide followed by HPV18 with 32.3% and 11.9% of adenocarcinoma and squamous cell carcinoma, respectively (Serrano, Brotons, Bosch, & Bruni, 2018). Almost 50% of entire HPV-related cancers in women is due to the carcinoma of cervix, while oropharynx-related cancers are frequently associated with HPV infection in men (Szymonowicz & Chen, 2020).

Between 70-90% of HPV-related cancers can be prevented by the immunisation of accessible prophylactic vaccines (Serrano et al., 2018). There are three commercially available HPV vaccines that are Cervarix (GlaxoSmithKline), Gardasil (Merck Inc.), and Gardasil 9 (Merck Inc.), which covers 2, 4 or 9 distinct HPV types, respectively. Even though the

preventive vaccine is applicable, the global burden of cervical cancer is not still relieved, especially in the underdeveloped countries. Therefore, scientists are still investigating novel approaches i.e., through targeting major HPV oncogenes E6 and E7 involved in carcinogenesis to develop new strategies to treat HPV-infection and its related cancerous lesions.

HPV GENOME AND ITS INTEGRATION TO HOST GENOME

7.9kb-long HPV genome contains 8 or 9 open reading frames (ORF) located on the same DNA strand and the genome can be separated into three distinct regions: 1- Early gene coding region (E1, E2, E4, E5, E6 and E7) 2- Late gene coding region (major, L1 and minor, L2 capsid proteins) 3- Non-coding region (or the long control region, LCR). Non-coding region or LCR consists of regulatory elements which are essential for viral DNA replication and mRNA transcription such as the replication origin and several transcription binding sites for RNA polymerase II, respectively. The 5′ end of DNA strand starts with the early gene sequences. E1 and E2 play a major role in the regulation of viral genome replication and transcription, whereas E5, E6 and E7 oncoproteins are crucial components leading to oncogenesis.

The life cycle of high-risk HPVs relies upon the differentiation mechanism of stratified epithelia. When the basal layer of the stratified squamous epithelial tissue is infected by HPV, DNA of the virus replicates itself and is maintained at low copy number by the functions of E1 and E2 proteins. Then, cells from basal layer start to differentiate and generate the epithelial suprabasal layer where the viral copy number is amplified followed by the expressions of late genes. Later, the virions are produced and released to infect neighbouring cells. Viral DNA may either be integrated to the host genome or preserved in an episomal status. Genome integration phenomenon was detected in more than 80% of HPV-associated cervical cancers (Cancer Genome Atlas Research et al., 2017). As various research has stated that the greater the occurrence of viral genome integration, the greater the seriousness of the cervical lesions, indicating the importance and necessity of this incident in tumorigenesis (Wentzensen, Vinokurova, & von Knebel Doeberitz, 2004). Following genome integration, E2 gene, which is given a paramount importance in the suppression of E6 and E7 expressions, mostly gets disrupted because of which E6 and E7 oncoproteins are expressed at higher levels.

HPV E6 AND E7 ONCOPROTEINS

HPV E6 and E7 are of grave significance in the process of tumorigenesis and cancer cell hallmarks including uncontrolled cell proliferation, inducing angiogenesis, activation of tissue invasion, metastasis, and telomerase activity as well as resisting apoptosis and evading tumour suppressors can be induced by the functions of these oncoproteins. Various studies in vivo and in cells have demonstrated that when the E6 and E7 are inhibited, senescence and apoptosis are induced, which indicate the need for continued expressions of E6 and E7 in HPV positive cancerous cells to maintain the cancer functions and phenotype (Cesur et al., 2015; Jabbar, Abrams, Glick, & Lambert, 2009; Nicol et al., 2013; Yamato et al., 2008).

HPV E6 and E7 are fairly small viral proteins consisting of nearly 150 and 100 amino acids, respectively, depending on the type of the virus. They do not function as enzymes, but form complexes with hosts' cellular proteins to manipulate their functions. There is an abundance of cellular proteins that are associated with E6 and E7 identified by interactome studies (White, Kramer, et al., 2012; White, Sowa, et al., 2012).

E6 and E7 oncoproteins interfere with the functions of p53 and pRb tumour suppressor proteins, respectively. Binding of E6 to host's ubiquitin ligase E6AP (E6-associated protein) results in the E6 conformational change. This alteration causes the generation of a new protein complex called E6/E6AP/p53 which then mediates the proteolytic cleavage of p53 (Martinez-Zapien et al., 2016; Scheffner, Werness, Huibregtse, Levine, & Howley, 1990). E7 binds to pRb and leads to its proteasomal degradation. Inactivation of pRb brings about the release of cellular E2F transcription factors leading to unplanned cell cycle progression that results from the subsequent expression of cell cycle inducing E2F target genes (Roman & Munger, 2013). When E6 and E7 are both expressed, cellular transformation occurs with high probability (Munger, Phelps, Bubb, Howley, & Schlegel, 1989). Namely, if E7 is expressed alone, it will have a proapoptotic effect as it induces p53; yet when E6 is also expressed it will inhibit p53 which then blocks apoptosis and causes outgrowth of deregulated cervical cancer cells (DeFilippis, Goodwin, Wu, & DiMaio, 2003). If E6 is inhibited in the presence of E7 expression, apoptosis will be induced in HPV transformed malignant cells (Belyaeva et al., 2014; Butz et al., 2003). In addition, oncogenic HPV types have effects on other p53- and pRb- dependent or independent mechanism that is important in cancer progression. For instance, E6 has a PDZ (PSD-95/DLG/ZO-1) binding

motif to interact with cellular proteins containing PDZ-binding domains such as potential tumour suppressors MAGI-1 and Scribble.

E6-mediated inhibition of p53 cause uncontrolled cell proliferation by escaping cellular checkpoints. In normal circumstances, if cells are under stress either in the shape of an oxidative damage or other types, the expression of genes engaged in cell cycle arrest or apoptosis will be induced. For cells to sustain continuous proliferation, p53 must be continually disrupted by E6. In addition, E7-mediated suppression of pRb is also crucial to maintain unrestricted cell proliferation. The pRb-E2F interaction in the G1-S phase transitions of the cell cycle constitutes a significant checkpoint. If cells are not ready for S phase transition, pRb protein remains bound to E2F transcription factor to inhibit transcription of genes needed for S phase entry. In HPV-positive cells, continuous expression of E7 give rise to the degradation of pRb, thereby releasing E2F and causing an immature entry to S phase. Together, E6 and E7 create the ideal setting to pass all anti-tumour checkpoints, resume proliferative signals and escape apoptotic protection. In addition, with the help of these oncoproteins, human telomerase reverse transcriptase enzyme (hTERT) (the catalytic unit of telomerase) is constantly expressed in cervical cancer cells providing replicative immortality. These proteins were also shown to trigger epithelial-mesenchymal transition (EMT), allowing tumour cells to metastasise to different parts of the body.

GENOME EDITING TOOLS TO TARGET HPV E6/E7

There is currently no effective treatment for persistent HPV infection. RNA interference is one of the partially advanced methods of genome editing using double-stranded RNA molecules called siRNA (small-interfering RNA) or shRNA (short-hairpin RNA). Targeting HPV E6 and E7 mRNAs by siRNAs impair their expression which subsequently leading to the accumulation of tumour suppressors such as p53, 21 and hyphophosphorylated pRb and induction of apoptosis in HPV-infected cancerous cells (Togtema et al., 2018). However, the effect of siRNAs is temporary as it is at the mRNA level and there will be no permanent impact unless the HPV DNA in the nucleus is degraded.

Lately, artificially designed genome editing techniques such as zinc finger nucleases (ZFNs), Tal-effector nucleases (TALENS) and the RNA guided engineered nucleases (RGENs or CRISPR/Cas9) have been employed to cleave HPV particular DNA sequences mainly E6 and E7 oncogenes for the

treatment of HPV infection. Targeting of viral genes whose expression is maintained exogenously is often preferred to avoid off-target effects which may occur by hindering the endogenous gene expression. Double stranded DNA breaks induced by these engineered nucleases cause the induction of DNA repair pathways (error-prone non-homologous end joining repair or more efficacious homology-directed repair pathway), contributing to the impairment of viral oncogenes and annihilation of HPV infection. Besides, by using genome editing tools in combination with a frequently used chemotherapy drug like cisplatin on the treatment of cervix carcinoma, the overall impact can synergistically be enhanced, which can be exemplified by some studies under clinical trials utilizing either ZFN, TALEN or CRISPR/Cas9 gene editing techniques (Ding et al., 2014; Hu et al., 2015; Hu et al., 2014; Zhen et al., 2016).

Zinc finger nucleases are synthetic nucleases generated by combining the DNA-binding zinc finger proteins with the FokI DNA cleavage region. Following binding to a DNA molecule, FokI nuclease is formed by dimerization of two artificially programmed ZFNs, generating a double strand break in the specific target DNA which then either cause cell apoptosis or induce DNA repair mechanisms. If DNA repair mechanism is pursued, non-homologous end joining will be preferred in which several mutations incorporate to the gene of interest, causing a regression of the disease and the reversal of histological malignant features. ZFNs were utilised to target E7 oncogene so that the HPV genome could be vigorously disrupted and as a result, augmentation of HPV 16/18 positive malignant cells was inhibited *in vitro*, and they were triggered to go through apoptosis (Mino, Mori, Aoyama, & Sera, 2013). Their therapeutic effects have also been investigated in xenograft mouse models *in vivo*, implicating their potential and effectiveness as an anti-cancer drug (Ding et al., 2014).

Like ZFNs, TALENs can also be utilised for the targeted therapy of HPV-related cancers through the cleavage of E6 and E7 of HPV16 and 18. ZFNs and TALENs are chimeric fusion proteins, both of which possess FokI nucleases. In addition, TALENs contain transcription activator-like effector DNA binding domain (30-40 nucleotides or more) while ZFNs are consisting of zinc-finger domain (18-24 nucleotide). It has been shown that unlike ZFNs, TALENs can effortlessly be engineered and are more effective in targeting HPV E6 and E7 oncogenes. With thriving editing of those oncogenes using TALENs, it was noted that apoptosis induced; tumour growth and thereby

tumorigenic effects were inhibited with an accompanying recovery in pRb levels in both HPV 16 positive and HPV 18 positive cell lines, that are SiHa and HeLa, respectively (Hu et al., 2015; Sumitra Shankar, Prasad, Sanawar, Das, & Pillai, 2017; S. Shankar, Sreekumar, Prasad, Das, & Pillai, 2018). Besides, gene editing was successfully accomplished through topical administration of polymer-complexed TELEN plasmids directly onto the cervix with malignant phenotype of K4-HPV16 transgenic mice (Hu et al., 2015). Results of the study were encouraging as cervical intraepithelial neoplasms (CIN) were converted to normal histological phenotype.

Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 is a straightforward system consisting of a single-guided RNA, directing Cas9 to the target sequence for the induction of double-stranded DNA breaks thereby disabling the gene. Therefore, it has a therapeutic potential for the therapy of HPV-related cervical malignancies. In fact, certain studies have demonstrated that the knockdown of E6 and E7 oncogenes was achieved by using CRISPR/Cas9 in HPV transformed cells, resulting in a hindrance in the cell proliferation, cell cycle arrest, apoptosis via restoration of pRb and p53 tumour suppressors, and eventually regression of the cancer progression (Kennedy et al., 2014).

This treatment protocol is not only limited to the cervix, therefore it needs to be further studied in other HPV-related such anogenital cancers as vulvar, anal, and penile cancers, in addition to head and neck cancers. Even though these highly sophisticated genome editing tools have strong therapeutic potential, they will not be readily available to everyone worldwide. Moreover, when their negative charge, low membrane permeability and large size are taken into consideration it is not yet explicit how these will be administered to the tissue of cervix (CerviPrep drug delivery system, vaginal suppository, gel, cream etc.) (Hodge et al., 2012). In addition, the viability of this high-cost therapy in the clinics seems highly debatable as cervical cancer often affects underdeveloped and poverty-stricken regions of the world.

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CHAPTER 8

TRANSCUTANEOUS AURICULAR VAGUS NERVE STIMULATION (taVNS)

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INTRODUCTION

The vagus nerve has a crucial role in maintaining autonomic tone of the brain structures and peripheral organs. The vagus nerve (VN) consisted of a mixed nerve that harbours afferent sensory fibers from the head, neck, abdomen and thorax. The term vagus nerve stimulation (VNS) in general means several different techniques using to stimulate the VN. Ta-VNS is a non-invasive and simple method that modulates the VN through the stimulation of the auricular branches of VN with few adverse effects. However, classical VNS requires surgery with resulting complications, limited accessibility and cost. Transcutaneous auricular vagus nerve stimulation (taVNS) is a novel method, which uses a stimulation device placed at the tragus or concha of the ear. It eliminated the need for surgery and is more accessible. Recent studies have focused on the use of taVNS in various diseases ranging from epilepsy to depression, obesity, glucose intolerance and cardiovascular diseases.

1. Vagus Nerve Anatomy

The vagus nerve (VN) has been traditionally considered a parasympathetic efferent nerve that control and regulate autonomic functions such as gastric tone and heart rate. The VN has a crucial role in maintaining autonomic tone of the peripheral organs and cerebral structures. However, VN actually composes of a mixed nerve that harbours afferent sensory fibers from the head, neck, abdomen and thorax. VN is composed of both sensory and motor fibers. It is the longest nerve that runs from the medulla to the colon and mainly innervates the abdominal and thoracic organs. The VN penetrates from the neck to the mediastinum, and travels posterior to pulmonary pedicle until reaching to the abdomen. Before it reaches the abdomen, VN forms cardiac plexus and peri-esophageal plexus, which provides origin to the anterior and posterior vagus nerves (Baccro, Lucas, Zandomeni et al., 2013).VN is involved in the cardiovascular, respiratory, gastrointestinal, autonomic, immune and endocrine systems (Yuan and Silberstein, 2016). Elements of the microscopic anatomy of the VN and most importantly the morphological features of the vagal fibers have been well-established in humans and other species (Jayaprakash, Song, Toth et al, 2022). The VN involves A-, B- and C-fibers described by Erlanger and Gasser inline with their conduction velocities that are proportional to the fiber sizes (Berthoud

and Neuhuber, 2000). Each fiber has its own physiological roles as described below**:**

- A-fibers: large myelinated A-fibers convey generally somatic afferent and efferent information, while small myelinated A-fibers mainly carry visceral afferent information. They also typically have diameters of around 1–22 μm and a conduction velocity of 5–120 m/s.
- B-fibers: supply efferent parasympathetic and sympathetic innervation with diameters = 3 μ m and a conduction velocity between 3-15 m/s.
- C-fibers: these are small unmyelinated fibers that provide afferent visceral information. They have diameters between 0.2 and 1.5 μm and a conduction velocity of 2 m/s.

Afferent fibers originate from receptors in the heart, aorta, lung and gastrointestinal tracts, while a small portion arise from the concha of the ear. Cell bodies of the afferent fibers are in the nodose and jugular ganglia and they project to the nucleus tractus solitarius, and nucleus cuneatus. Efferent fibers provide parasympathetic innervation mainly to the heart, lung and gastrointestinal tract as well as innervate the voluntary muscles in the pharynx and larynx. These fibers arise from the preganglionic neurons in the dorsal motor nucleus of the VN.

Most of the VN fibers are afferent C-fibers that travel from the visceral organs. The cervical branch vagus nerve is made up of about 20% myelinated A and B fibers and 80% unmyelinated C fibers (Vonck, De Herdt and Boon, 2009). Vagal fibers run through fascicles and form branches that innervate organs and regulate organ function. Studies conducted on cats have shown that the VN involves 80% afferent fibers traveling from the visceral organs to the nucleus tractus solitarius (Foley and DuBois, 1937; González, Yengo-Kahn, A and Englot, 2019). There is an asymmetrical innervation to the heart. Whereas the right VN innervates the sinoatrial node, the left VN innervates the atrioventricular node.

Positron-emission tomography and functional MRI examinations on the effects of VN stimulation (VNS) on humans have confirmed the major role of the VN on higher brain sections. These studies have shown decreases

and increases im blood flow as a response to VNS (Henry TR, Bakay RAE, Votaw JR, et al., 1998; Henry TR, Votaw JR, Bakay RAE, et al., 1999).

2. Vagus Nerve Stimulation (VNS)

The term VNS in general means several different techniques using to stimulate the VN, including animal studies in which the vagus was accessed through the diaphragm and abdomen. For human studies, VNS refers to stimulation of the left cervical VN using commercial devices. For some, VNS is an option before brain surgery (George, Sackeim, Rush et al., 2000).

The VNS therapy system consists of a pulse generator, a bipolar VND lead, a tunneling tool, a programming wand and hand-held magnets (LivaNova, 2018). The generator transmits electrical signals to the VN through the lead. The software enables insertion of the programming wand over the generator to read and alter stimulation parameters. Each stimulation period is preceded by 2 s of ramp-up time followed by 2 s of ramp-down time (Wheless, Gienapp and Ryvlin, 2018).

VNS therapy is approved for the treatment of epilepsy without age or seizure limitations in more than 70 countries worldwide. As of June 2018, more than 100,000 patients have received VNS therapy all over the world (Wheless, Gienapp and Ryvlin, 2018). The FDA has approved the use of implantable VNS for the treatment of drug-resistant epilepsy and depression. In recent years, studies have reported promising results in treating several diseases including headache, Alzheimer's disease, sepsis, anxiety, lung injury and diabetes (Ben-Menachem, Revesz, Simon et al., 2015). However, this classical technique requires a surgical intervention in the cervical trunk of VN, and thus its use is limited by the possible risks of surgery, availability and costs (Fang, Rong and Hong, 2016).

2.1. The history of VNS

Scientists have been interested in whether and how autonomic functions modulate activity in the higher cortex and limbic system for many years. VNS was first developed in the late 19th century by James Corning, an American neurologist, for the treatment of epilepsy (Yuan and Silberstein, 2016) A large number of studies have identified extensive projections of the VN through its sensory afferent connections in the nucleus tractus solitarius to many areas of the brain. In a study by Bailey and Bremer on cats, it was

reported that VNS elicited synchronized activity in the orbital cortex (Bailey and Bremer, 1938). Dell and Olson discovered that VNS caused a slow-wave response in the amygdala in awake cats with high spinal section (Dell and Olson, 1951). Based on these initial studies, Zabara showed that VNS had a anticonvulsant action on experimental seizure model in dogs (Zabara 1985). Based on this finding, Zabara concluded that VNS could prevent or control the motor, autonomic, and conscious components of epilepsy. According to Zabara, VNS had two different mechanism of action:

1. A direct inhibition of beginning or ongoing seizure
2. A long-lasting inhibition to prevent seizures

These successful results in animal models have encouraged researchers to assess the feasibility of using VNS in human subjects with neurological conditions. The modern use of VNS in humans was introduced by Kiffin Penry et al. in 1988 with the first human implant for the treatment of epilepsy (Penry and Dean 1990). Since that time, five generations of VNS therapy system technology have been introduced.

3. Transcutaneous Auricular Vagus Nerve Stimulation (taVNS)

VNS has traditionally required the implantation of a programmable device and electrodes that stimulate afferent fibers of the VN directly. This procedure is performed under general anesthesia and requires a neck incision and dissection to the VN where three electrodes are inserted (Giordano, Zicca, Barba et al., 2017).

It has been shown in the anatomy studies that the ear is the only place on the surface of the human body with afferent VN distribution (Henry, 2002; Peuker and Filler, 2002). There is a direct projection from the auricular branch of the VN to the nucleus tractus solitarius (NTS). Anatomical studies conducted on the ear suggest that the tragus, concha, and cymba concha are the places on the human body where there are cutaneous afferent vagus nerve distributions (Peuker and Filler, 2002), and it is believed that stimulation of these afferent fibers should produce therapeutic effects that are similar to those of regular VNS (Hein et al., 2012; Rong et al., 2012). Based on this information, direct stimulation of the afferent nerve fibers found on the ear could produce an effect similar to classic VNS in reducing seizures (He,

Rong, Li et al., 2012). With the development of a noninvasive method of VNS through transcutaneous stimulation of the auricular branch of the VN, there is now a safer and better tolerated method for performing VNS (Redgrave, Day, Leung et al., 2018). Transcutaneous auricular vagus nerve stimulation (taVNS) is a method, which uses device that stimulates the tragus or concha of the ear. It eliminates the need for and it is more accessible. In recent years, taVNS studies have focused on the treatment of epilepsy, pain, migrain, schizophrenia, depression, Parkinson's disease, induced atrial fibrillation and impaired glucose tolerance (Johnson and Wilson, 2018).

In animal studies equivalent antiseizure effects of taVNS and direct cervical VNS have been observed (He, Zhu, Rong, 2009). ta-VNS stimulates the thick and myelinated sensory Aβ-fibers in the VN, and activates the NST (He, Jing, Zhu et al., 2013). Functional imaging performed during taVNS has been shown to have a pattern compatible with the stimulation of the vagal nerve (Frangos, Ellrich and Komisaruk, 2015). Ta-VNS is a non-invasive and simple method that modulates the VN through the stimulation of the auricular branches of VN with few adverse effects (Fang, Rong, Hong et al., 2016). The literature on taVNS is growing fast because of the availability of resources. Today, taVNS is a fast growing technology with implementations in bioelectronic medicine. TaVNS has been used for the treatment of numerous diseases including neurodegenerative diseases, inflammation, cardiovascular diseases and chronic pain (Kaniusas, Szeles, Kampusch et al., 2019).

It has been shown in a recent study that taVNS could be used in acute respiratory distress syndrome due to COVID-19 (Kaniusas, Szeles, Kampusch, 2020). Baig et al. demonstrated improvements in upper limb sensation following taVNS paired with motor training in the majority of stroke survivors (Baig, Falidas, Laud et al., 2019). Rong et al. reported that ta-VNS is a promising, safe and cost effective alternative treatment method for Major Depressive Disorder (MDD) (Rong, Liu, Wang et al., 2016). However, studies using taVNS are not consistent in terms of the stimulation parameter sets, making comparison of the studies on this issue challenging.

3.1. TaVNS Technique

Although may differ among various studies, in a typical taVNS technique, the electrodes are positioned either to the cymba concha or tragus of the ear in a supine position. Stimulation electrodes are made of a round conductive metal combined with a conductive medium such as electrolyte gel.

The taVNS stimulator may be either a battery driven device or powered from a conventional electrical outlet. A programmed computer software is used to control the stimulator and initiate stimulation with specific parameters, including intensity, pulse width, frequency and session duration.

The patient sits on a comfortable bed in a supine position with legs elevated and head supported. The left ear is inspected and the stimulation target is located. A thin layer of conductive paste is spread to the surface of the electrodes. The anode electrode is inserted inside the ear canal, targeting the anterior wall of the outer ear canal. The cathode electrode is located on the outside of the ear attached to the tragus. For sham stimulation, the anode is inserted on the anterior side of the ear.

Perceptual threshold is determined using a simple step-up and step-down binary parametric search. First the stimulator is turned on and the output is set to 3 mA. One second train of taVNS stimulation is delivered at desired pulse width and frequency. A computer-generated pulse is used to drive the stimulation system. This interface software/stimulator interface allows modulation of frequency, duty cycle (on/off time), and session duration. Stimulation is delivered at super-threshold levels such as 200 PT. When stimulation is completed, objective data regarding stimulation discomfort and side effects are recorded. The stimulation electrode is removed from the ear and residual paste is cleaned. The ear is inspected for redness or irritation at the stimulation area (Figure 1) (Badran, Yu and Adair, 2019).

Figure 1. Stimulation locations. Black points show the taVNS.

3.2. Ta-VNS in treatment of Epilepsy

Epilepsy is one of the most common neurological disorders and is defined as recurrent epileptic seizures. Approximately one third of epilepsy patients needs comprehensive treatment efforts in order to obtain seizure-free state or at least an acceptable seizure state (Kwan & Brodie, 2000). An epileptic seizure is defined as a transient development of signs and symptoms because of excessive neuronal activity in the brain (Fisher, DesMarteau, Koontz et al., 2020). The prevalence of epilepsy has been reported between 0.5 to 1% with about 50,000 patients worldwide (World Health Organization, 2019). For some patients with epilepsy (PWE), resective epilepsy surgery is an option for seizure freedom or reduction. For those who are not eligible for surgery or not willing surgery, alternative treatment methods are necessary. One of these alternatives is taVNS. It was demonstrated in a proof of concept trial with taVNS that seizure frequency was reduced over 9-month follow-up in 5 of 7 patients. Tolerability was rated as 'good' to 'very good' in 6 of 7 patients (Stefan, Kreiselmeyer, Kerling et al., 2012). In a prospective pilot trial with 14 children that had epilepsy, efficacy of tavNS was demonstrated in 7 of 13 children of which 4 were seizure free after 4 months (He, Jing and Wang, 2013). In a single randomized controlled study over 12 months, 60 children and adults were randomly assigned to the treatment group and control group. Seizure frequency was significantly reduced after stimulation in the treatment group compared to the baseline values after 6 and 12 months ($p<0.001$) (Aihua, Lu and Liping et al., 2014).

TaVNS studies in epilepsy reported promising results in PWE with improved seizure control and severity. Quality of life (QoL) was also improved in most patients. Adverse effects were mild-to-moderate and mostly tolerable.

3.3. TaVNS in treatment of Major Depressive Disorder

MDD is a widespread life-threatening disease characterized by impaired cognition, tendency to suicide, reduced energy level, and vegetative symptoms (Marchetti, Koster and Sonuga-Barke et al., 2012). The most commonly used treatment options in MDD include antidepressant drugs, psychotherapy, deep-brain stimulation, cognitive behavior therapy. However, only 35% of these patients respond to medication. According to the theory underlying taVNS, the VN plays an important role in relationship between gut, spleen, brain and inflammation (Fox, 2017). It is thought that taVNS is linked to the microbiome-brain-gut axis that regulates the relationships

between brain regions mediating antidepressant effects. It has been postulated that taVNS can significantly decrease the symptoms of depression such as cognitive impairment, depression, anxiety, sleep disturbance etc. taVNS has anti-inflammatory properties that are exerted through activation of the hypothalamic−pituitary−adrenal axis, the cholinergic anti-inflammatory pathway, and brain regions or circuits in MDD (Liu, Yang and Zhang, 2020).

4. CONCLUSION

TaVNS is yet its infancy and is under development with new advancements every passing day. TaVNS has shown promising results in patients with epilepsy with improved control and frequency of seizures. The number of diseases treated through taVNS is increasing, including Alzheimer's disease, Parkinson's disease, headache, migrain, depression, anxiety, sepsis and pain management. However, the relationship between the stimulation parameters from taVNS devices and effectiveness is yet to be elucidated. Further comprehensive studies in this area are urgently needed.

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CHAPTER 9

CANCER SCREENINGS IN THE FAMILY MEDICINE SYSTEM IN TURKEY

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INTRODUCTION

This chapter defines the cancer screenings in Family Medicine system in Turkey. Cancers are defined as a malignant tumors that occur as a result of abnormal growth of cells in the organism. In fact, cancers are a broad spectrum that causes the emergence of hundreds of diseases by capturing the immortalization of cells in different parts of the human body. It can cause serious disease or even death if left untreated. Cancer is still the world's and our country's second greatest reason of mortality, after cardiovascular illness. In 2018, cancer killed nearly 10 million people globally. This shows that cancer kills one in every six deaths worldwide. On the other hand, according to the International Agency for Research on Cancer (IARC) organization, an affiliated organization of the World Health Organization (WHO), one out of every five people in the world is diagnosed with cancer during their lifetime. The table 1 lists the top ten most common cancers globally for both genders.

Table 1. Global Cancer Incidence: Both Gender

Rank	Cancer	New cases in 2020	% of all cancers
	All cancers*	18,094,716	
1	**Breast**	2,261,419	12.5
2	Lung	2,206,771	12.2
3	**Colorectal****	1,931,590	10.7
4	Prostate	1,414,259	7.8
5	Stomach	1,089,103	6.0
6	Liver	905,677	5.0
7	**Cervix uteri**	604,127	3.3
8	Oesophagus	604,100	3.3
9	Thyroid	586,202	3.2
10	Bladder	573,278	3.2

* Except for non-melanoma skin cancer; contains “other and undetermined cancers” cases (not included here)

** Quantified by addition colon cancer and rectal cancer cases

As can be seen, breast, colorectal, and cervical cancers, which are included in screening programs, are among the top ten malignancies worldwide. In Turkey, the Turkish Cancer Control Program was prepared for the first time in 2008. And then the first national statistics report was designed in 2014 with the efforts of the Public Health Institution Cancer Registry

Center. TUIK 2017 data show that cancer killed 81,527 persons in Turkey, and this constitutes 19.6% of all deaths in Turkey. The projection data of the incidence of cancer in Turkey between the years 2017-2023 show us that in the following years there will be a rise (Figure 1).

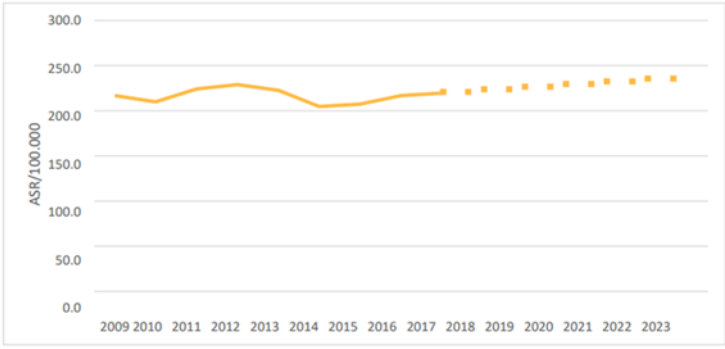

Figure 1. Prediction of Cancer Incidence in Turkey Between 2017 and 2023

When compared with the GLOBOCAN data of 2020; Cancer incidence in Turkey is slightly above the world incidence. Turkey 2017 national data; showed that nearly 65% of cervical cancers were Phase 0, about 50% of female breast cancers were Phase 0-1, and almost 40% of colorectal cancers were in Phase 0-1 (Figure 2).

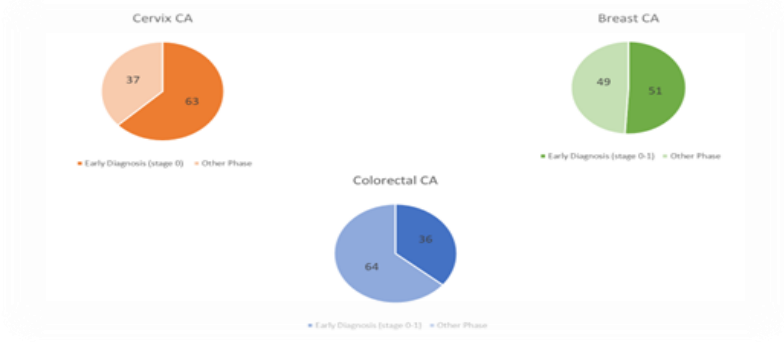

Figure 2. Phase Distributions of Screening Cancers in Turkey (Turkey Combined Database, 2017)

This figure is valuable in demonstrating the importance of scanning programs. As in the world, breast, colorectal and cervical cancer screening and cancer screening programs are implemented in our country.

1. BREAST CANCER SCREENING

Breast cancer is a prominent health trouble in Turkey as well as around world. The most frequent type of cancer among women is breast cancer. National cancer statistics show that breast cancer is diagnosed in one out of every four female cancer patients in Turkey, with the median age at diagnosis is 53. The data for 2020 showed us that while approximately 2.5 million women worldwide are living with breast cancer, unfortunately almost a quarter of them will die. And regrettably in the same year 604,000 new cervical cancer cases were diagnosed and 342,000 deaths occurred. According to World Health Organization (WHO) IARC (International Agency on Cancer Research) data, there were almost 2 million new breast cancer cases globally in 2018. The distinction between lung cancer and breast cancer, the most common type of cancer included in the same report, was only 5000 cases. While the incidence of breast cancer in Turkey was around 50/100,000 in 2018, the number of newly diagnosed breast cancer cases was recorded as 22,500. In Turkey, it is the most frequent type of breast cancer (35.6%) in women aged 25-49 (35.6%), age 50-69 (26.3%), and women aged 70 and over (14.7%). A systematic approach has been adopted in breast cancer screening procedures in the Family Medicine system. (Figure 3)

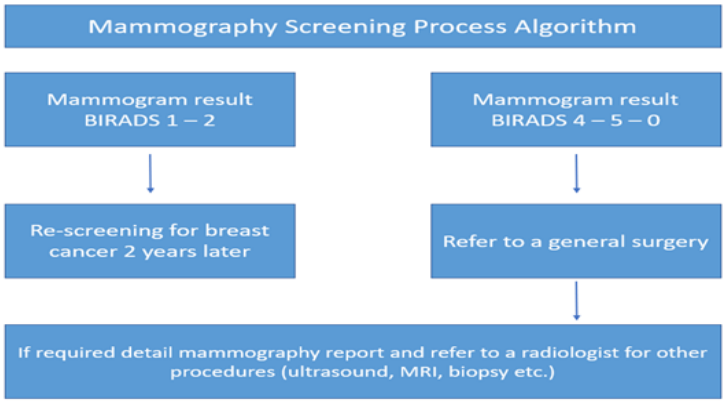

Figure 3. Algorithm in Breast Cancer Screening Procedure

Here, the target population is women between the ages of 40-69 who are registered with a family physician. A patient invited by a family physician;

- An anamnesis is taken and a physical examination is performed
- Risk factors for breast diseases are questioned

- Counseling services are provided or directed to screening centers to perform breast self-examination once a month
- Physical examination of breast is inspected by the physician once a year or referred to screening centers.
- Information is given about breast cancer and mammography, and they are referred to the relevant center.

Individuals with normal mammography results are given a second appointment after 2 years. Patients with suspicious or abnormal mammography results are referred to the relevant hospitals. All steps of the screening process for the patient are recorded in the AHBS system by the family physician. In summary, in a breast cancer screening:

- Women's breast self-examination: A monthly breast self-exam is recommended for women aged 20 and over.
- Office examination: It is the breast examination performed by the doctor. This process is done every two years for those over 20 years old and every year for those over 40 years old.
- Mammography: It should be done every two years for women between the ages of 40-69.

2. CERVICAL CANCER SCREENING

Today, cervical cancer is still the 4th most frequent malignancy in women globally. Almost 80% of cervical cancer deaths occurring in emerging countries such as Turkey. Cervical cancer screening protocols have been in operation for a long time in many countries, especially in developing countries and have been proven to be beneficial in reducing mortality. The main purpose of screening for cervical cancer;

- Implementation of a national screening program to be established throughout the country to the target population
- Detection of cervical pathologies while they are still premalignant or at an early stage
- To prevent possible complex and expensive treatments by reducing the frequency of invasive cancer, its associated morbidity and mortality, by treating it with effective and simple methods HPV (Human Papilloma Virus) test or Papanicolau test (PAP Smear) is applied to women aged 30-65 every 5 years for cervical cancer screening in the Family Medicine system in Turkey.

2.1. HPV Testing

The relationship between HPV DNA and cervical cancer has now been established, and the presence of HPV DNA has been verified in 99.9% of cervical cancer patients. If the HPV test is negative; the probability of developing cervical cancer in the following five years is very low.

2.2. Pap-smear Testing

The Pap-smear test is a screening test based on detecting tumor cells at an early stage for classifying by searching for abnormal cervical cells in swab. This cytological screening test detects non-symptomatic preinvasive and early invasive cervical lesions. In Turkey, cervical cancer screenings are performed by the Cancer Early Diagnosis, Screening and Training Centers [in Turkish: KETEM], Healthy Living Centers, Family Health Centers, and Community Health Centers.

However, in practice, scanning procedure is mostly done by health personnel who are trained in taking smears in KETEM. The population to be screened is determined by the family physician. A family physician invites certain people from the population registered to him to the screening process, which is repeated every 5 years. Figure 4 shows the path taken during the scanning procedure in cervical cancer.

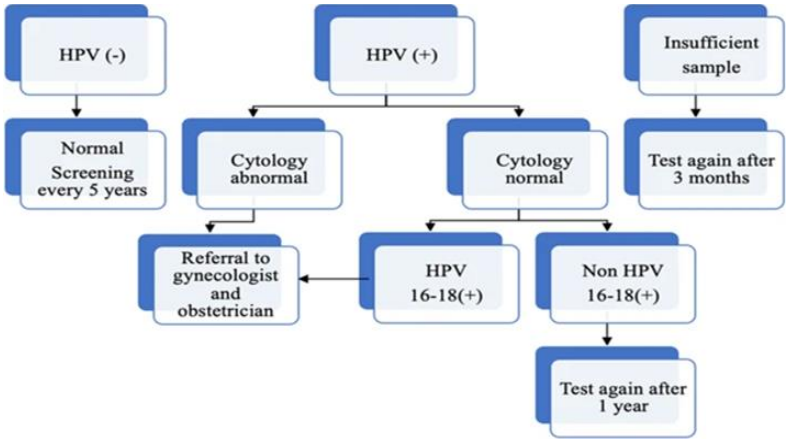

Figure 4. Algorithm in Cervical Cancer Screening Procedure

Those who are found to have abnormal cytology as a result of the pap-smear test or who are positive for HPV 16/18 test are directed to the obstetrician for further examination and evaluation.

3. COLORECTAL CANCER SCREENING

Colorectal cancer is a common clinical condition, impacting over one million people worldwide each year. Colorectal cancers remain the third most common malignancy in both men and women. It affects 25.1men per 100,000 and 14.7 women per 100,000. Colorectal cancer correlates strongly with increasing age, with the highest rate over 75 years and the lowest under 40 years of age. Men and women aged 50 to 70 should be the target population for colorectal scanning programs. Colorectal cancer is one of the most prevalent cancers among men aged 25 to 49 in our country. Screening tests are used to both prevent and detect colorectal cancer at an early stage. Figure 5 illustrates the procedures of screening of colorectal cancer.

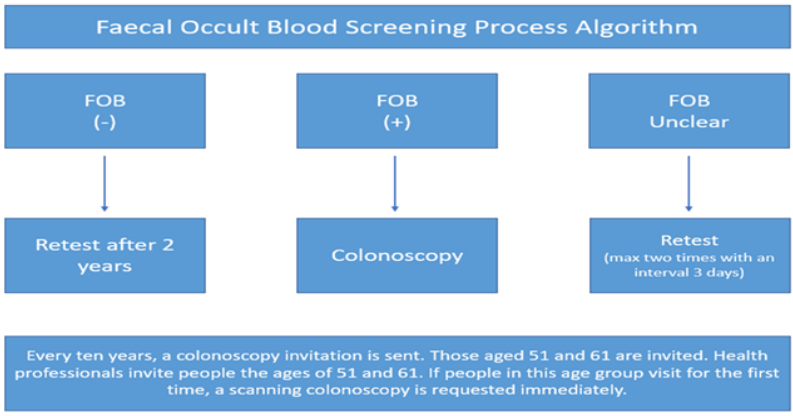

Figure 5. Colorectal Cancer Screening Algorithm

Feacal occult blood test, recto-sigmoidoscopy, colonoscopy and imaging modalities are applied if required in routine screening.

3.1. Faecal Occult Blood (FOB) Testing

In Turkey, it is recommended to apply every 2 years to men and women between the ages of 50-70. Men and women over 70 year-old who have had negative stool occult blood tests in the last two years should no longer be screened.

3.2. Colonoscopy

It is recommended to be done every 10 years for men and women aged 51 – 61. The population registered in the family medicine system, which

is included in the screening program, is given an appointment in two-year periods. The screening procedure is finished if the results of two consecutive test were negative in idividuals over 70-year-old, whether female or male.

CONCLUSION

Screening programs can detect premalignant adenomatous polyps, cervical diseases, and early stage breast cancer patients. By reaching the target population, it is possible to diagnose and treat early localized malignancies. Morbidity and mortality will be minimized when these simple and effective procedures are supplemented with appropriate treatment. A health reform has been established in our country as a result of the National Cancer Control Program. Public participation should be assured by screening program awareness research, and the efficacy and inclusivity of cancer screening programs should be increased across the country. Integrating cancer screening into the Family Medicine system as a part of preventive health services and supporting Family Doctors in this field will always maintain its critical importance for public health.

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CHAPTER 10

NATURAL KILLER CELLS AND CANCER IMMUNOTHERAPY

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INTRODUCTION

The specific method used in the treatment of diseases such as cancer by using immune system cells is called immunotherapy. Immunotherapy is also called biological therapy or biotherapy. Recently, the treatment method for diseases such as cancer has been moving from non-specific methods to specific ones. Tumor cells are destroyed using traditional treatment methods. However, spreading to other tissues with metastasis is an important problem. Although traditional methods and combined drug therapy successfully kill cancer cells, they also damage healthy cells. Therefore, the importance of immunotherapy-based treatment approaches is increasing (Wayteck, et. al. 2014). In immunotherapy, an immune response can be created against cancer cells with substances given externally to the body's immune cells. For example, NK (Natural Killer) Cells, T lymphocytes, B lymphocytes, macrophages, and dendritic cells may also play a role in tumor destruction. Unlike chemotherapeutics, these immune cells directly target cancer cells (Barbaros and Dikmen 2015).

1. IMMUNE SYSTEM AND CANCER

The immune system is a complex system consisting of several cells, cytokines, and proteins that are responsible for protecting the organism against external and internal threats. In addition, the immune response is the recognition of the foreign substance by the host and the creation of a response (Akcam, 2005). The immune system protects the body against cancer, which may be caused by environmental factors and genetic factors, or against infections that may be caused by microorganisms such as viruses, bacteria, fungi, and parasites.

The immune system is divided into two groups “natural immunity” and “acquired immunity”. The body creates the first natural immune response against a foreign antigen. It then introduces slower-acting adaptive immune mechanisms. Primarily, phagocytic cells such as neutrophils and monocytes, NK cells, cytokines, and some plasma proteins act against a pathogen or genetically modified cells. Then, T and B lymphocytes, which are among the adaptive immune elements, come into play. Innate immunity is a non-specific system that first responds to pathogens that enter the body. Acquired immunity, on the other hand, is a system that produces a more specific response, with a greater antigen recognition capacity, but a slower response. Innate and adaptive immunity work together to form a robust defense system

to protect the host against foreign cells or various intracellular and extracellular pathogens. (Figure 1)

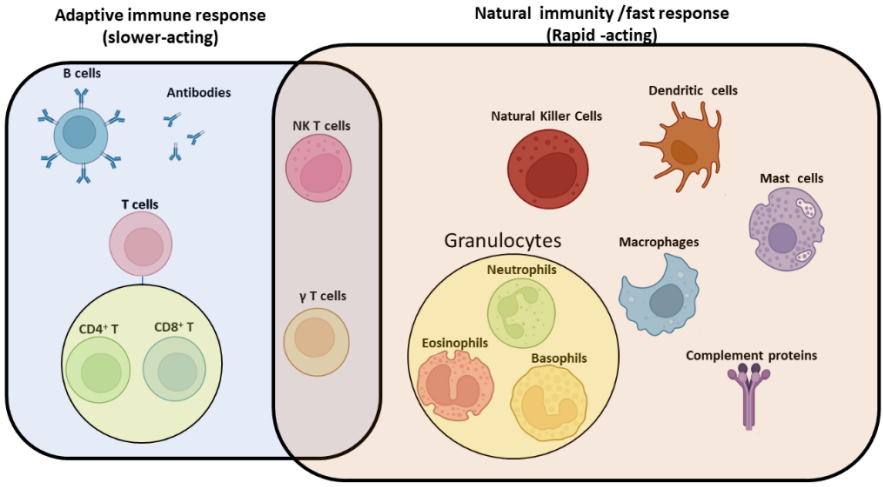

Figure 1: Natural immunity and adaptive immune response in the body

The immune system has two roles in fighting cancer cells. The first of these is the immune surveillance mechanism. Here, the immune system recognizes and destroys cancer cells. This process is carried out by pro-immune humoral factors that increase in the tumor microenvironment, such as natural killer cells, natural killer T cells, T cells, and interferon, which are the main cells of the innate and adaptive immune system. In the second, while the immune system tries to destroy the cancer cells, the cancer cells also cause changes against the immune system.

Immune regulation consists of three stages discussed below:

Elimination: In this phase, the immune system is active and dominant against cancer cells. Cancer cells that can escape from this phase continue to multiply and move toward the next phase, the equilibrium phase. Growing cancer cells migrate toward the tissues, and with the occurrence of damage, an immune response begins to occur (Grivennikov, et. al. 2010). Natural killer cells (NK), dendritic cells, macrophages, and T cells from immune system cells gather here to produce INF gamma (IFN-γ) (Barbaros and Dikmen 2015). With its apoptotic and antiproliferative effects, IFN-γ causes the destruction of cancer cells and the release of chemokines into the

environment. Chemokines prevent vessel formation in tumors and allow more macrophage cells and NK cells to come to the medium (Balkwill, 2004; Tanaka, et. al. 2005). Tumor-destroying NK cells and macrophages interact, causing more IFN-γ and interleukin-12 production. With the release of tumor necrosis factors (TNF) into the environment, perforin, ligand that increases apoptosis, and reactive oxygen are released, causing the death of more cancer cells (Barbaros and Dikmen 2015).

Equilibrium: This phase is called the immune surveillance mechanism. In this phase, the cancer cells escape the elimination phase and the immune system are in a dynamic balance. In the equilibrium phase, both the tumor and the immune system are active. While the immune system tries to recognize and destroy cancer cells, cancer cells also try to make the tumor microenvironment suitable for themselves with the cytokines they produce. Cancer cells neutralize the immune response by forcing immune cells to some transformations (T helper2, Treg, MDSC, tumor-associated macrophages) with cytokines, such as transforming growth factor-beta (TGF-β), interleukin-10 (IL-10), prostaglandin E2 (PGE2). They try to suppress the inflammatory response. The equilibrium phase can continue for a long time, and eventually either the immune system succeeds and destroys the cancer cells or the cancer cells prevail and begin to multiply.

Escape: In this phase, which is the escape phase, cancer cells have been effective against the immune system. The tumor turned the immune system in its favor and started to grow uncontrollably and spread to other tissues (Dunn, et al. 2004).

2. NK CELLS

NK cells, one of the important cells of the immune system, recognize cancer cells or infected cells and destroy them directly. One of the important features of NK cells is that they do not have a memory. Therefore, they recognize and act on cancer cells that lack MHC Class I molecules. The activity of NK cells occurs through the binding of the Fas receptor on the surface of cancer cells and the Fas ligand on the surface of NK cells (Yılmaz, 2019). In addition, NK cell activity is regulated by the inhibitor and activator molecules they carry. With activation, exocytosis of perforin-granzyme-containing granules and cytokines occurs. The most known natural killer cells are immunoglobin-like receptors (Killer cell immunoglobulin-like receptor, KIR) (Gras Navarro, et al. 2015).

NK cells, also called natural killer cells, are a type of lymphocyte derived from hematopoietic stem cells in the bone marrow. These cells mediate cytotoxicity against the cancer cell and virally infected cells (Ames, et al. 2009; Ames and Murphy 2014; Cheng, et al. 2013). They are the specialized defense cells of the immune system and make up 10% of the lymphocytes in the blood (Sun and Lanier 2011). These cells, which play important roles in both innate and adaptive immune systems, enable the activation of monocytes and cytotoxic T cells and also play a role in the maturation of dendritic cells. They also stimulate or suppress B cells to produce immunoglobulin. Cytokines produced by NK cells also affect T helper cell polarization (Deniz, et al. 2013).

As stated, they play an important role in the general immune response. NK cells do not attack cells that normally express MHC-I (Major Histocompatibility Complex-I) molecules, but they attack and kill cells that have decreased or absent MHC-I expression. This is seen in cancer cells or viral infections. In addition, NK cells can regulate other immune system cells by secreting interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) and stop the proliferation of target cells (Zingoni, et al. 2005). The most common lysis mechanism of cytotoxic NK cells is their secretion of lytic granules.

NK cells use three different mechanisms to kill cancer cells. These are the following:

- They secrete lytic granules containing perforin and granzymes. Perforin creates pores in the cell membrane, allowing the passage of granzymes, while granzymes, which are a family of serine proteases, induce caspase and apoptosis pathways in cancer cells.
- In the second mechanism, Fas ligand (FasL) and Tumor Necrosis Factor (TNF)-related apoptosis-inducing ligand-TRAIL (TRAIL-TNF-related apoptosis-inducing ligand) induce apoptosis and lead to cell death via death receptors on the cell surface.
- In the third mechanism, NK cells act on cancer cells by secreting IFN-γ and TNF-α cytokines. With the release of these cytokines, NK cells not only affect tumor cells directly but also modulate macrophages, dendritic cells, and T cells. They are immune regulatory cells that can affect anti-cancer responses (Liu, et al.

2007). Secretion of IFN-γ provides activation of macrophages and dendritic cells (Scott 1991). TNF-α release causes necrosis of cancer cells (Brinkman, et al. 1995)

3. EFFECT OF NK CELLS ON CANCER CELLS

3.1 NK Cells Lead to Death by Apoptosis Pathway:

NK cells cause death by affecting the apoptosis pathways of cancer cells using death receptors and lytic granules containing perforin-granzyme B, without causing any necrotic effect. The apoptosis pathway is divided into two intrinsic and extrinsic (Rapoport and Anderson 2019).

Internal pathway: Perforin and granzymes secreted from immune cells affects the intrinsic pathway. They are activated by various microenvironmental stressors such as intrinsic pathway DNA damage in apoptosis, reduction of growth factor inducing mitochondrial dysfunction, ER stress, calcium overload, and replication stress (Rapoport and Anderson 2019). The intrinsic pathway of apoptosis is controlled by the Bcl-2 protein family (Zaman, et al. 2014). Pro-apoptotic stimuli only initiate up-regulation of Bcl-2 homology 3 (proapoptotic Bcl-2 protein containing only the BH3 domain). Later, these proteins activate proteins (Bax, Bak, etc.) that will form pores in the mitochondrial membrane (Liu and Zhu 2017; Lomonosova and Chinnadurai 2008). These oligomerize, increasing the permeability of the mitochondrial outer membrane. This is the most important event occurring in the inner pathway (Elmore, 2007). Subsequently, cytochrome-c is released from mitochondrial membrane proteins. Cytochrome-c release triggers APAF-1 (Apoptotic protease activating factor-1) and procaspase (Hassan, et al. 2014; Zaman, et al. 2014; Green and Llambi 2015). As a result, cell death occurs with the loss of mitochondrial membrane (Van de Walle, et al. 2008).

Extrinsic pathway: Death receptors on the membrane trigger the extrinsic pathway. These include Fas ligand, TRIAL (TNF-related apoptosis-inducing ligand), and TNF (Tumor necrosis factor) (Goldar, et al. 2015). After ligand-receptor binding, an adapter protein is taken into the medium (Zaman, et al. 2014; Liu, et al. 2017). FADD (Fas-associated death domain) and TRADD (Receptor-associated death domain) are the two most common adapter proteins. 8 and 10 of the initiator pro-caspases bind to the adapter protein to form the death-inducing signaling complex (DISC), resulting in the activation of the pro-caspases. This activation provides activation of caspases

3, 6, and 7, which mediate the division of intracellular proteins and lead to cell death (Zaman, et al. 2014).

NK cells, which are immune effector cells, are activated by the cytokines INF-γ, IL-2, IL-12, IL-15, and IL-18. In addition, survival and maturation processes take place through these cytokines (Nahta, 2012). After activation, the polarization of the granules against the target cell occurs, and the lytic granules are released out of the cell and cause the target cell to be lysed. In other words, NK cells cause cell death by activating apoptosis in cancer cells. They are resistant to the proteases contained in their granules. Chondroitin sulfate-A, a negatively charged proteoglycan in NK cells, prevents NK cells from autolyzing themselves (Delves, et. al. 2008). In addition, NK cells can specifically target cancer cells and, unlike chemotherapeutic drugs, they can directly target cancer cells to kill them (Barbaros and Dikmen 2015). They respond directly to transformed cells either by cytotoxicity or by secreting cytokines (Pegram, et al. 2011). Cytotoxic cell lines such as YT, NKG, NKL, NK-92, and KHYG-1 have been generated from malignant NK cell clones. NK-92 cells have the most cytotoxic potential of the NK cell subgroups. In addition, NK-92 cells show significant cytotoxic activity against cancer cells in preclinical studies. It is the only cell line approved for use in scientific studies by the FDA (Tonn, et. al. 2013). NK-92 cells have been shown to kill K562 myeloid leukemia and Daudi lymphoma cells by 84% and 86%, respectively (Gong, et al. 1994). In addition, it has been stated that NK-92 cells are suitable for clinical development (Schönfeld, et al. 2015).

4. INHIBITOR AND ACTIVATOR RECEPTORS ON NK CELLS

NK cell cytotoxicity is mediated by a variety of inhibitory and activating cell surface receptors. The balance between these receptors determines the activation or inhibition of NK cells (Pegram, et al. 2011). Two different types of NK receptor families have been identified. These are Ig-like receptors and C-type lectin receptors. Both receptor structures contain activating and inhibitory receptors. Inhibitory receptors contain structures called ITIM (Immunoreceptor tyrosine-based inhibitory motifs) at their cytoplasmic ends. These motifs detect certain phosphatases and inhibit signal transduction. When inhibition does not occur, NK cells become activated and secrete cytokines and cytotoxic granules. Activating receptors are associated with proteins such as DAP-12. These are ITAM (Immunoreceptor tyrosine-

based activator motifs) motifs showing positive activation at the cytoplasmic end (Delves, et al. 2008).

a) Activator Receptors: Cells transform and express cellular stress ligands on their surface when they become cancerous. The activator receptors on the NK cell surface recognize the stress ligands expressed on the tumor cell surfaces, and they realize that these cells are defect cells and show anti-cancer effects. Major NK cell activator receptors are CD16, NKG2D, DNAM-1, activating KIRs, and natural cytotoxicity receptors (NKp30, NKp44, and NKp46).

b) Inhibitory Receptors: Cancer cells express some molecules against the inhibitor receptors on the surface of NK cells to protect themselves from the anti-cancer effect of NK cells. In this way, they suppress NK cell cytotoxicity and are protected from the anti-cancer effect. The main inhibitory receptors on NK cells are killer cell immunoglobulin-like receptor-KIR (Killer-cell Immunoglobulin-like Receptor), leukocyte immunoglobulin-like receptor-LIR (Leukocyte Immunoglobulin-like Receptor), PD-1, TIM-3, TIGIT, and CD94/NKG2A. (Table 1)

Table 1: Activator and Inhibitor Receptors on NK Cells

Inhibitor	Activator
KIR2DL1/2/3 (HLA-C)	KIR2DS1/S2 (HLA-C)
KIR3DL1(HLA-B)	KIR2DS4 (HLA-A, -C)
KIR3DL2(HLA-A)	KIR2DL4 (HLA-G, HS)
CD94/NKG2A(HLA-E)	CD94/NKG2C (HLA-E)
LIR-1(HLA-A-G)	KIR3DL2 (CpG)
KLRG-1(cadherins)	CD16 (IgG)
CEACAM1(CEACAM5)	NKG2D (MIC, ULBP)
TIGIT (PVR, PVRL2)	NCRs (B7-H6, NKp44L)
Coreceptor	**Coreceptor**
Siglec -3 -7 -9 (sialic acid)	DNAM-1 (PVR, Nectin-2)
LAIR-1(collagen)	2B4 (CD48)
CD300-A (PS)	NTBA (NTBA)
	NKp80 (AICL)

While some subgroups of the KIR receptor family play an inhibitory role, some play an activator role (Leung, 2014). KIR, LIR, and heterodimer CD94/NKG2A on NK cells are important classes of inhibitor receptors. These receptors are involved in the recognition of normal cells by immune cells. In addition, cancer cells can use the immune system escape route by mimicking normal cells. These receptors are located on a fine line between the two states. The recognition or escape mechanism between immune cells and cancer cells begins in this situation. As soon as the NK cell detects the cancer cell as foreign, it increases its activator receptors, secretes lytic granules and cytokines, and tries to lead the cell to apoptosis through the FasL death receptor. Cancer cells, on the other hand, try to suppress the expression of NK cell activator receptors by expressing NK cell inhibitory receptor ligands

(such as HLA-A, HLA-E, etc.) and suppress NK cell activity by secreting TGF-β. In the fight between the NK cell and the cancer cell, the party that demonstrates more effectiveness is successful. For this reason, disrupting this balance and making NK cells more advantageous against cancer cells is a promising approach to offer a new treatment model and increase treatment efficacy.

5. MECHANISM OF NK CELLS TO RECOGNIZE CANCER CELLS

In the immune system, NK cells are tolerant and do not harm healthy cells. It can recognize and kill defective cells. NK cells have developed various mechanisms to differentiate healthy cells and cancer cells. These mechanisms form the basis for NK cell activation. All healthy cells are protected from the killing mechanism of NK cells by expressing the major histocompatibility complex MHC-I (Major Histocompatibility Complex) molecules. They express normal levels of HLA-I (Human Leukocyte antigen-I) molecules that match inhibitor receptors KIR or CD94/NKG2A on NK cells (Romagne, et al. 2009). Inhibition receptors stop NK activity by interacting with HLA-I molecules, a subgroup of MHC-I molecules (Shah and Shpall 2009). NK cells recognize cancer cells in two ways:

- Cells transform and lose HLA-I molecules. However, the inhibitory signals on NK cells, which have inhibitory KIRs or CD-94/NKG2A receptors, do not get into the cells, but rather NK cells are activated and increase their lytic granules and cytokine release. This model is called "Missing-self recognition" (Ljunggren and Karre 1990).
- NK cells express activating receptors that recognize stress-induced ligands on the surface of transformed cells. As the cell transforms and becomes cancerous, the expression of damage-related proteins increases. These proteins, whose expression is increased, can combine with activating receptors on NK cells and increase the cytotoxicity of NK cells. This model is called “stress-induced recognition” (Mosser, et al. 1997; Groh, et al. 1998).

The balance between activating or inhibitory signals on the NK cell influences the response of NK cells to cancer cells. When this balance shifts towards activation, NK cells directly or indirectly kill cancer cells. On the

contrary, when the inhibition predominates, NK cells stop their anti-cancer ability (Le Mercier, et al. 2015).

NK cells play a role in the body's first defense system against cancer cells. It recognizes and destroys cells that have transformed. However, cancer cells express HLA-I molecules like healthy cells to escape the immune system. Cancer cells manage to stop the activity of these cells by binding to NK cell inhibition receptors. Therefore, preventing the escape of tumor cells from the first defense system is an important point for treatment. For this reason, the first approach applied for cancer treatment with NK cells; is the administration of activating molecules such as interleukin-2. In this way, the activation and persistence of NK cells are increased. The secondary approach is; includes cytokines to kill tumor cells, antibodies specific to inhibitor and activator receptors, and adoptive transfer of NK cells (Ljunggren and Malmberg 2007). Different combinations are tried with these treatment methods, and the search for more effective NK cell-based treatment models for each type of cancer continues. In several studies; The anti-cancer potential of NK cells has been successfully exploited against neuroblastoma (Yang et al. 2013) and glioblastoma (GenBler, et al. 2016; Han, et al. 2015; Zhang, et al. 2015). In addition, promising findings for lung cancer have been reported (Tonn, et al. 2013; Besse, et al. 2016). For this reason, NK cell-based cancer immunotherapy is currently the focus of attention because of its rapid and specific effects only against cancer cells without damaging healthy cells. NK cells are cytotoxic against cancer cells and activate caspase and apoptosis pathways. It is also a good option for therapeutic use without leaving any toxic effects, like drugs.

6. RECENT ADVANCES IN NK-BASED CANCER IMMUNOTHERAPY

Cancer immunotherapy, which has become an important step in oncology today, is a treatment method in which the body uses its immune system to fight cancer cells. This approach uses the strength and selectivity of the immune system for treatment by inducing the cancer patient's immune system cells (Disis, 2014; Shirkhoda, et al. 2018). Cancer immunotherapy aims to stimulate the immune system against cancer cells and make immune cells more effective and defensive. It also activates the immune system by exposing components such as immune system proteins. In recent years, immunotherapy has become an important part of treatment for some types of

cancer. It is thought that the treatments performed with the immune system will guide the cancer treatment approaches in the future. Table 2 shows the NK therapy timeline.

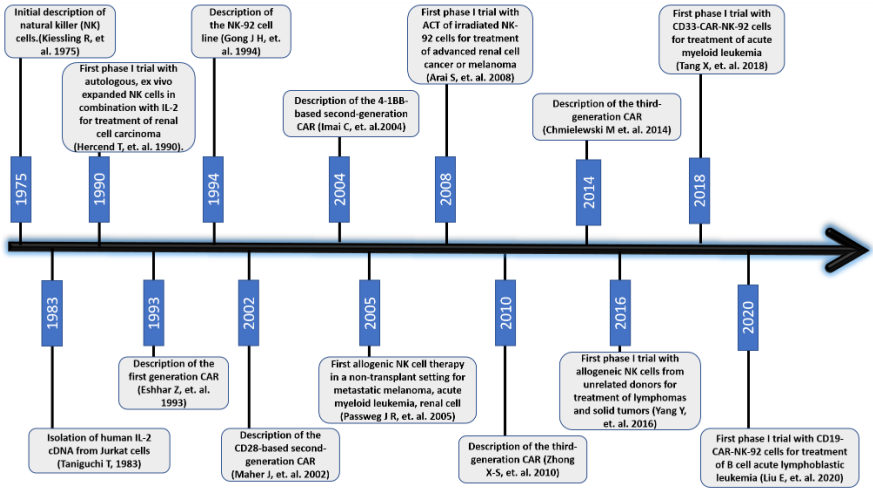

Figure 2: NK therapy timeline

Cancer cells express antigens that make them look like normal cells to evade the immune system. In this way, they escape from the immune system. Sometimes, because cancer cells secrete immunosuppressive factors such as TGF-β (Tumor Growth Factor), an adequate and strong response may not be produced by NK cells. To overcome such effects, studies have found ways to affect activator and inhibitor receptors through antibodies, which will help the immune system recognize cancer cells and strengthen its response. These antibodies have been approved by the FDA for use in therapy (Stojanovic, et al. 2014; Westin, et al. 2014; Huang, et al. 2015; Lesokhin, et al. 2015). Drug candidates consisting of antibodies and molecules that will identify and target immune system checkpoints continue to be investigated.

Below are the main types of immunotherapy used for cancer treatment:

a. **Monoclonal antibodies:** Monoclonal antibodies, which are human-made immune system proteins, are immunoglobulins with a targeted glycoprotein structure. All of the therapeutic monoclonal antibodies used in the clinic are antibodies of the immunoglobulin G (IgG) isotype (Saeed and Awan 2016). The main mechanisms of action of

IgG antibodies are i. complement-dependent cytotoxicity, ii. antibody-dependent cellular cytotoxicity and phagocytosis, iii. disruption of receptor-ligand interaction, iv. receptor internalization or degradation, and v. cell death by induction of apoptosis (Strohl and Strohl 2012).

b. **Immune checkpoint inhibitors:** These types of drugs help to identify and destroy cancer cells by removing the blockages created by cancer cells in the immune system. Immune checkpoints provide escape by blocking the response of immune cells against cancer cells. Primary antibodies such as PD-1, PD-L1, and CTLA-4 are used in the clinic to eliminate this immunosuppression. In this way, the use of monoclonal antibodies that strengthen the immune system against tumor cells has significantly improved the prognosis in patients (Ansell, et al. 2015; Garon, et al. 2015; Bellmunt, et al. 2017). Dr. James Allison and Dr. Tasuku Honjo received the 2018 Nobel Prize in Physiology and Medicine for their work on anti-CTLA-4 and anti-PD-1 monoclonal antibodies and immune checkpoints in cancer.
c. **Adoptive immunotherapy:** It is called adoptive immunotherapy when active cytotoxic immune cells are multiplied in vitro for cancer treatment and prevention of tumor formation (Özet, et al. 1996). NK and T's cells are used in adaptive immunotherapy. TIL (tumor-infiltrating lymphocytes) are cells regulated for chimeric antigen receptor (CAR) expression, which combines the extracellular portion of the antibody and the signaling mechanism of NK and T cell receptors (Barbaros and Dikmen 2015). Although this system gives important results in hematological and solid tumors, further optimization, increasing effectiveness, and reducing toxicity are required (Oiseth and Aziz 2017; Rohaan, et al. 2019).
d. **Cytokines:** These are polypeptides that transmit signals to cells. With cytokine binding, intracellular signals are triggered as a result of changes in gene transcription at receptors. These signals enable to stimulate or change the differentiation, proliferation, and cell functions. Therapeutically, they act with a direct pro-apoptotic or antiproliferative effect or indirectly by increasing the cytotoxic activity of immune cells against cancer cells (Berraondo, et al. 2019).
e. **Cancer vaccines:** They are used to initiate the immune response against diseases. They aim to treat advanced cancers. Some cancer vaccines are for prevention, while others are for treatment.

New immune cell-based treatment models are being researched that will prevent the growth and spread of cancer cells and target specific points (Gardner, et al. 2016; Parlar, et al. 2019). These immunotherapy approaches are still being investigated for every cancer type as an exciting approach, one of the current focuses is to make NK cells, which can find and kill defective cells in the immune system, most effective for cancer treatment.

CONCLUSION

In conclusion, NK cell immunotherapy is a promising field in cancer treatment that harnesses the power of the body's immune system to fight cancer cells. By using natural killer (NK) cells, a type of immune cell, to target and destroy cancer cells, this therapy aims to provide a safe and effective alternative to traditional cancer treatments. Recent advancements in technology have enabled the engineering of NK cells to enhance their cancer-killing abilities and increase their specificity for cancer cells. This has resulted in the development of various NK cell-based immunotherapies that are currently being tested in clinical trials for a wide range of cancers. Despite its potential, the field of NK cell immunotherapy is still in its early stages and more research is needed to fully understand its potential benefits and limitations. However, the results so far are promising and the use of NK cells as a tool in cancer treatment holds great promise for the future.

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CHAPTER 11

EVALUATION OF THE EFFECTS OF SOCIOECONOMIC APPROACHES AND PSYCHOLOGY İN İNFERTİLİTY

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INTRODUCTION

Infertility is defined as the absence of pregnancy despite regular unprotected sexual intercourse for one year. It is stated that one out of every ten couples in the world has a problem with infertility. Since having a child is perceived as the aim of marriage and family institution in almost every society. For couples, having a child is accepted as a biological, psychological, social and cultural need (Mosher & Pratt, 1991). Reproduction and perpetuation are one of the most important and basic instincts of all living things. Infertility is a complex situational crisis that is often psychologically threatening, emotionally stressful, economically expensive, and often physically painful for both partners due to diagnostic and therapeutic procedures (Şirin, 2001). Infertility is a life-changing experience that brings with it medical, psychiatric, psychological and social problems, has cultural, religious and class aspects, compares it with individual and unexpected stressors, results in social labeling, causes feelings of sexual failure and inadequacy (Guz, Ozkan, Sarisoy, Yanik, & Yanik, 2003). Although infertility is not classified as a life-threatening disease, it is a social problem that affects the individual, family and society. It is a devastating and therefore an important health problem that causes individual, family and social problems (Ozcelik, Karamustafalıoğlu, & Ozcelik, 2007). In infertility, the age of the woman is a dangerous factor. The number of women who have their first child after the age of 35 is increasing. With the increase in the education level of women, the increase in employment opportunities, career development delays the age of marriage and the age of birth. It is reported that one in five women in the United States have their first baby after the age of 35 (Ozkan & Baysal, 2006). There are many biopsychososal and cultural factors that cause infertile women to experience psychological problems. The fact that a woman of childbearing age learns that she cannot give birth to a child unexpectedly without a biological reason despite her will, is experienced as a serious loss, a painful experience, and disrupts the psychological and social life of her husband as well as the woman. The loss of the sense of motherhood brought about by the inability to become pregnant causes social stigma, especially in traditional societies where the social status of women is determined by being a mother, and impairs women's psychological health (Karaca & Unsal, 2015). In addition, infertility is a complex and uncertain process that affects the lives and psychology of couples in many ways after it enters their lives, they do not know how long it will be included in their lives

from the moment they are learned, and if they choose to resort to treatment, it is devastating in terms of the sense of mother and father that they lost before they could get it (Özcelik, Karamustafalioglu, & Özcelik, 2007). As a result of the psychological, social and sexual problems and pressures experienced, the problem of adjustment of couples increases, conflicts arise, and this makes infertility and violence a phenomenon that needs to be discussed. The World Health Organization (WHO) reported in its report published in 2002 that violence mostly occurs in the family environment and against women (Krug, Mercy, Dahlberg, & Zwi, 2002). Motherhood is perceived as the primary duty of women in the eyes of the society, and in the case of childlessness, the woman is blamed and oppressed in the first degree. The woman, who is shown to be primarily responsible by her social environment, her husband's family and her own family, is more likely to encounter both social and domestic violence. Although research on infertility and domestic violence has been carried out in many countries, the literature has focused on women, and studies on men and couples are still limited. Akyüz et al., in their study with 228 infertile and 204 fertile women in 2013 on the effect of marital violence on women's infertility distress in Turkey, found that the mean severity score of the infertile group was higher than the fertile group. Infertile women have higher levels of sexual, economic and emotional violence compared to fertile women. In the same study, most of the infertile women stated that their spouses humiliated and humiliated them because of their infertility (Akyüz, Sahiner, Seven, & Bakır, 2014). A study of infertile men in Africa showed that men are also exposed to psychological and social violence, even if not physical, 27 infertile men participated in the study and they explained that they and their spouses were socially humiliated, they knew they were talked about behind their backs, and they were exposed to humiliating jokes in social environments (Dyer, Abrahams, Mokoena, & van der Spuy, 2004). However, in general, women are abused more in the family, especially in the case of infertility, and society sees infertility as a woman's problem. In studies on the reason for wanting children in the family; While love, happiness and satisfaction of maternal instinct come first, as a secondary reason; the continuation of the marriage. While the demand for children is at the forefront in women; The compulsion of the sanction of social pressure as parents is more central in men. In a study conducted in Sweden on this subject, the most common reason expressed by women is; While it is a sign of love between spouses, the most common reasons men show; it was determined that the addition of a new member to the family and the child's main meaning of life

(Lalos, Jacobsson, Lalos, & von Schoultz, 1985). The most common emotion experienced by individuals diagnosed with infertility is sadness. Other feelings accompanying childlessness; surprise, denial, anger, bargaining (if I get pregnant..), unhappiness, introversion, isolation, mourning, guilt, worthlessness, frustration, acceptance. In a study, the dominant emotions in 31% of women and 16% of men; sadness, pessimism, despair; It was determined that 23% of women and 16% of men felt lonely (Kamacı, 2003). The relationship between infertility and psychological signs and symptoms has been investigated in many studies. Most of the studies reveal that there is a serious relationship between infertility and psychological symptoms. It was determined that the greatest psychological difficulty in infertile patients was anxiety, and depression in unsuccessful couples. In a study of 112 women to determine the prevalence of psychological stress in infertility patients, 40% met criteria for a psychiatric disorder. The most common diagnosis is anxiety disorder (23%). This was followed by major depressive disorder (17%) and dysthymic disorder (9.8%). These findings reflect the higher prevalence of psychiatric disorders in infertility cases, with an average prevalence of 10-12% (Chen, Chang, Tsai, & Juang, 2004).

Infertility and Social Problems

Studies in this area say that the way men and women perceive the pressure experienced by infertile couples and how they react differ. While depression and anxiety problems are observed more in women, it is mentioned that fear of stigma, inadequacy and loss of power are more intense in men. The pressure exerted by the social environment can also cause the couple to become isolated and withdrawn. While women are more comfortable talking and sharing with their friends, men prefer not to talk about this issue more. Just as men and women react differently in psychological processes, their social treatment and reactions are also different. In the case of infertility, the woman is more likely to face social stigma and face questions, while male infertility is perceived as a situation that needs to be covered (Oğuz, 2004). Defined as a life crisis, infertility creates a crisis situation not only for couples but also for families of women and men. While having a child is a status and success in the society, childlessness negatively affects the perception of femininity and masculinity, together with failure in the social context. As a result of this influence; exclusion, social violence, forced divorce, and second marriages are on the rise. While family meetings and meeting with friends were a part of life and fun before the diagnosis, these activities turn into a

source of pain and stress after being diagnosed with infertility. The questions asked at these meetings, the curiosity that violates the privacy of the child, cause the couples not to attend such meetings and interviews and to isolate themselves socially over time (BAYRAKTAR, 2018). The social support provided by the family, close environment and society of the person and the couple has a very important role in coping with stress. Even if social support does not eliminate the stress factor, infertility, it enables couples to decrease their stress level, lower their anxiety, be more optimistic, have self-control and try new ways to cope with stress.

The Effect of Infertility on Sexual Life

Other psychiatric diseases frequently seen in infertile patients; sexual dysfunction, somatization disorder, dysthymia, panic disorder, obsessive compulsive disorder and social phobia. Eating disorders such as anorexia nervosa, bulimia nervosa, and obesity have also been associated with infertility. Alcohol and substance abuse can also be seen. While increased anger and aggression severity was detected in infertile women, There are different studies that detect anger inward or outward. In patients undergoing infertility treatment; It has been emphasized that hormonal imbalance in the hypothalamo-pituitary-ovarian axis or hormonal drugs used may cause mood disorders (Klemetti, Raitanen, Sihvo, Saarni, & Koponen, 2010). Studies on sexual problems show that with the emergence of infertility, sexual function problems also occur due to increased stress and pressure. Interviews with the participants of the study support the literature. Although infertility is a common problem of partners, male and female partners may give different emotional reactions in case of infertility. In comparative studies, it was observed that clinical depression and anxiety (anxiety) were less common in males. More psychological problems (depression and anxiety) in women are explained by the fact that they are more exposed to medical tests and the hormones they take for treatment cause some psychological changes. Couples perceive the procedures applied in the diagnosis of infertility as fault-finding events, feeling of being watched and optional sexual intercourse (such as post-coital test and semen analysis), and these situations may result in deterioration in marital relations, decrease in sexual desire and performance. In comparative studies, it has been determined that women in infertile couples experience more stress than men, and it has been reported that men are less affected by infertility emotionally and psychologically (Abbey, Andrews, & Halman, 1995). Infertility is considered a serious psychological and

relationship stressor, and many experts report that there is a link between infertility and sexual dysfunction. In the presence of infertility, sexuality lags behind reproductive function, leading to a decrease in sense of self, feeling of inadequacy, guilt, and the emergence of many problems that spread to many areas of life (Burns, 2006). Since infertility affects the marital relationship, deteriorations in sexual function and satisfaction usually follow. In studies evaluating the relationship between infertility and sexual dysfunction, it has been reported that infertile men experience a significant decrease in sexual intercourse frequency and satisfaction. In one of them, the prevalence of ED and andropause symptoms was found to be 28% in infertile men and 11% in fertile men (O'Brien, Lazarou, Deane, Jarvi, & Zini, 2005). In a study comparing 18 infertile men and 12 fertile men who applied for sterilization, it was found that the rate of sexual dysfunction and sexual satisfaction was lower in infertile men; It has also been reported that there is a tendency to decrease in sexual desire (8). Other studies have also reported a decrease in the frequency of ED and sexual intercourse in infertile men. In another study investigating the prevalence of sexual dysfunction in infertile couples, 100 infertile couples were evaluated and only one third of the men had a normal or higher IIEF score.

Emotional Stages of Infertile Couples

As the literature reveals, couples who learn that they are infertile go through some emotional stages after being diagnosed. These are associated with the grieving process and are listed as shock, denial, anger, bargaining, depression, and acceptance.

Stage 1 is the shock stage. The behaviors that most of the participants describe when they learn that they are infertile show how the shock phase was experienced.

Stage 2 is the stage of denial. In the denial phase, the couple is intertwined with the thoughts that this could not have happened to us. Disbelief in examinations and changing doctors are defense mechanisms specific to this stage.

Stage 3 is the anger stage. This stage shows the anger and guilt felt by the couple or the individual, a person responsible for the situation is sought and the anger is directed there.

Stage 4 is the bargaining stage. During this period, the couple or the person expects miraculous changes and wants to change the reality they deny. Bargaining is the phase in which bargaining phrases such as "If I have a child, I will sacrifice", "If I have a child, I will not commit a sin again" are used the most.

Stage 5 is the depression stage. It is the stage where pain and grief are experienced most intensely and the couple sees the truth with all its nakedness. In this phase, the couple becomes withdrawn, lonely, and does not want to see anyone. Especially avoid families with children, pregnant friends or relatives.

Although the emotional phases of infertile couples are seen in all individuals, it takes a long time for some couples and individuals to reach the dissolution phase; it has been seen that some couples and individuals are stuck at one stage and still experience the crisis situation caused by infertility.

Table 1. The relationship of fertility status and some variables

Variable		**Fertility status**		**x^2**	**P**
		Infertile	**Fertile**		
Menstruation	regular	308(60.9)	790(79.9)	50.989	0.001
	irregular	201(40.5)	225(23.2)		
Ability to conceive a child	Yes	164(34.12)	908(92.3)	5.950	0.001
	No	89(9.2)	328(68.8)		
Infertility among relatives	Yes	156(31.8)	330(35.7)	1.970	0.167
	No	349(70.2)	632(66.8)		

Table 2. Comparison of mean of variables in fertile and infertile women

Variable	Mean ± SD		t	P
	Infertile	**Fertile**		
Age at menarche	14.42±1.85	14.60±1.90	-1.60	0.178
Duration of marriage (years)	9.84±7.44	11.36±7.57	-3.98	0.001
Age	32.04±7.89	32.58±6.92	-0.999	0.338
Spouse's age	36.88±9.37	36.99±7.58	-0.235	0.820
Age at first marriage	22.09±5.51	21.78±4.64	-3.342	0.001
Attitude	57.64±7.23	57.89±6.76	-0.577	0.579

Table 3. Comparison of interventions made by the relatives of the two groups

Type of intervention		Fertility status		x^2	P
		Fertile	**Infertile**		
		N (%)	**N (%)**		
Encouraging divorce	No	1042(69.2)	499(33.2)	19.57	0.001
	Yes	6(32.7)	19(78.3)		
Encouraging remarriage	No	1052(68.9)	488(33.1)	52.92	0.001

Type of intervention		Fertility status		x^2	P
		Fertile	**Infertile**		
		N (%)	**N (%)**		
	Yes	6(23.2)	38(89.8)		
Encouraging adoption	No	1054(69.8)	487(32.9)	87.98	0.001
	Yes	5(7.3)	48(97.8)		

DISCUSSION

Infertility is a long and difficult process for couples and individuals that is not known when it will end and is difficult to cope with. Every marriage brings with it the expectations of becoming a parent, getting pregnant, and getting pregnant, and chaos prevails in the lives of people who unexpectedly learn that they are infertile. It was observed that all of the couple and single individuals participating in the study experienced all phases of the vital crisis model proposed by Menninge (1977), which was explained in the conceptual framework. It is an important point to be underlined that some participants are stuck in a phase and have not yet passed the dissolution phase. (Menning, 1988). As in most societies, in our society, the child is perceived as an important part of the family, and the desire of the woman to be a mother is reinforced. This perception can turn into a psychological and social pressure that makes it compulsory to bear children and become a mother in married women. Childlessness is a cause of social stigma, especially for women. In addition, repeated and unsuccessful treatments can trigger anxiety and depression symptoms. In a study conducted with women who received in vitro fertilization treatment, in which the effects of anxiety and depression on conception were measured, it was reported that high levels of anxiety adversely affected pregnancy outcomes. In infertile couples, both hormonal problems due to the side effects of the drugs used in the treatment and psychological problems due to psychosocial factors affect pregnancy rates negatively (Meyers et al., 1995). In a study by Demyttenaere et al., they found

that depressive symptoms were associated with low pregnancy rates in women receiving infertility treatment. In another study, it is stated that there is a significant increase in pregnancy rates along with a decrease in depression and anxiety levels (Demyttenaere et al., 1998). With the emergence of the effects of the psychological problems experienced by women struggling with infertility on the treatment process and pregnancy, it has been reported that the number of studies investigating the effects of psychosocial interventions on infertile couples should increase and risky groups should be determined by screening for anxiety and depression before treatment (Wischmann, 2003). Considering the rates of depression and high level of anxiety in our study, it can be argued that psychosocial interventions, which will be handled with a holistic and multidisciplinary approach, should be used as a part of infertility treatment. In this study, there was an inverse relationship between education level and anxiety and depression scores; It was determined that as the education level decreased, the depression and anxiety scores increased. In a study conducted by Aliyeh and Laya with infertile women, a similar result was found, and it was reported that women with low education levels had higher depression scores. In a study, it was reported that women with low education levels are more affected by the stigma of infertility and are more exposed to psychological problems (Link & Phelan, 2001). In another study, it was determined that infertile women with low education level and not working had lower quality of life and insufficient coping with psychological problems (Teskereci & Oncel, 2013). In our country, the rate of women in the 15-49 age group who have a high school or higher education level is around 41%. Only 28% of these women of childbearing age work in an income-generating job. It can be predicted that women with a high level of education may have higher levels of household welfare, benefit more from health services, and respond to social pressures with richer coping resources, since they generally work in an income-generating job where they can provide economic freedom. Although it is a remarkable finding that depression and anxiety scores are high in infertile women, comparative studies with larger sample groups are needed to determine the reasons for this. In future studies investigating the psychological effects of infertility treatment, it is recommended to conduct studies with control groups that target homogeneous groups in terms of socio-demographic characteristics such as years of vulnerability, education level and employment status.

CONCLUSION

Infertility; It is a life crisis that brings along psychosocial problems. Taking preventive measures by addressing psychosocial problems that may affect the success of treatment is an important issue that should be emphasized in the delivery of health services. Knowing the psychosocial problems experienced by individuals during the infertility treatment process can facilitate the adaptation of infertile individuals to infertility and treatment and reduce their reactions to infertility. Infertility is not a simple gynecological disease, but affects women negatively, especially biologically, psychologically and socially; As a result, it is a condition that reduces health and quality of life. Psychological symptoms that affect the current medical treatment of patients and the course of their disease can often escape the attention of clinicians or be misdiagnosed. It is important to examine the cases from a biopsychosocial point of view and to support the search for psychiatric help. With inter-clinical cooperation, it is possible to increase the quality of life of patients, reduce treatment costs, and reduce wasted time for the treatment team and patients. The first diagnosis, examination, treatment, learning that the treatment is negative, abortion, etc. at different stages of the treatment. It is recommended that further studies be conducted to evaluate the level of individuals affected by infertility.

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CHAPTER 12

ACUTE MESENTERIC ISCHEMIA

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INTRODUCTION

Acute mesenteric ischemia (AMI) is an umbrella term involving four main pathophysiological mechanisms including mesenteric artery thrombosis (MAT), mesenteric artery embolism (MAE) , mesenteric vein thrombosis (MVT) and non-occlusive mesenteric ischemia (NOMI). These mechanisms lead to ischemia/reperfusion syndrome of the bowel, which is responsible for the occurrence of multiple organ failure and mortality if not treated promptly (Abboud, Daher and Boujaoude, 2008). AMI mainly affects patients over 60 years old with male predominance with an incidence of 1/1000 hospitalizations. Its rate of hospital mortality has been reported as 5% (Ravipati et al., 2011). The growing elderly adult population is the main factor affecting the increasing incidence of AMI. Despite the advances in knowledge of pathophysiology, laboratory investigations and imaging modalities, AMI is a potentially fatal vascular emergency largely because of the difficulty in making a timely diagnosis. The rate of mortality is over 60% if the diagnosis takes more than 12 hours and 90% if the diagnosis takes a longer time (Menke, 2010; Florian et al., 2010). High clinical suspicion, properly selected imaging modalities to establish the diagnosis and knowledge of the factors increasing surgical efficacy are the main principles for an efficient management of AMI. In general, severe and diffuse periumbilical abdominal pain creates a high index of clinical suspicion for AMI. Since the symptoms and results of the laboratory testing are not specific, imaging investigations play an important role in the diagnosis of AMI. Treatment of AMI includes restoration of the blood flow using an endovascular or surgical approach and resection of the necrotic intestine part. In this chapter, etiology, risk factors, pathophysiology, clinical presentation, diagnosis and treatment of AMI are discussed.

1. Etiology

Acute mesenteric ischemia may develop due to a disruption in arterial or venous mesenteric circulation. The most common etiology of AMI has arterial origin and includes MAT and MAE. Less common causes of AMI are venous and include MVT and low-output stages such as NOMI. In addition, vasculitis, arterial dissection, mesenteric trauma, volvulus, adhesions and internal hernias are the other causes of AMI (Florim et al., 2018).

Superior mesenteric artery (SMA) is the most commonly involved vessel in AMI. SMA embolism is the most common form of AMI by 50%

followed by SMA thrombosis, NOMI and MVT (Lock, 2001; Borioni et al., 2021). Etiologies of AMI are shown in Figure 1.

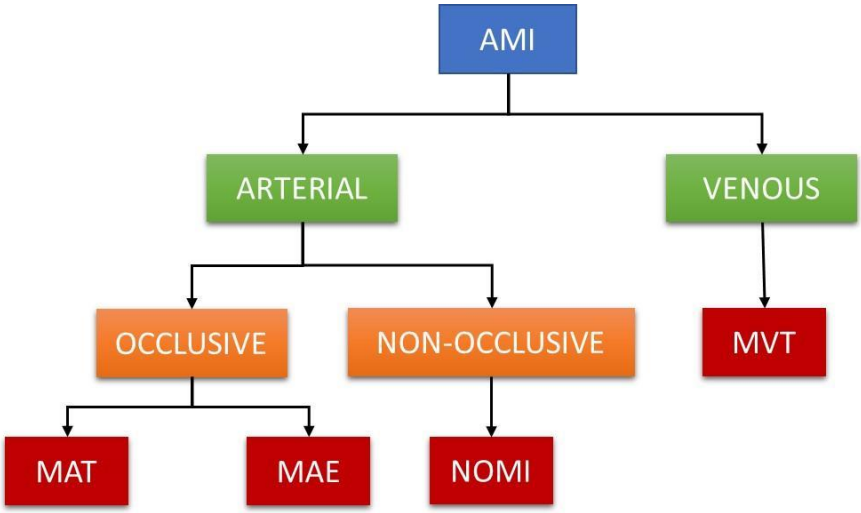

Figure 1. Causes of AMI
AMI: acute mesenteric ischemia, MAT: mesenteric artery thrombosis, MAE: mesenteric artery embolism, MVT: mesenteric venous thrombosis, NOMI: non-occlusive mesenteric ischemia

1.1. Mesenteric Artery Thrombosis (MAT)

MAT is a condition in which the arterial vascular supply of the intestinal system is occluded. MAT is in general a complication of previously existing stenosing atherosclerosis of visceral arteries. Mesenteric infarction symptoms tend to develop subacutely because of the formation of collaterals. MAT is a potentially fatal disorder of superior mesenteric artery (SMA), which supplies the small intestine and ascending colon (Ehlert, 2018). A history of weight loss and postprandial abdominal pain suggests chronic mesenteric ischemia (Gnanapandithan and Feuerstadt, 2020).

1.2. Mesenteric Artery Embolism (MAE)

MAE is a rare and urgent acute abdominal emergency with a very high rate of mortality and is difficult to be diagnosed early (Liao et al., 2019). MAE demands prompt diagnosis and a coordinated multidisciplinary management. Superior mesenteric artery embolism (SMAE) accounts for about half of the causes of AMI (Li et al., 2022).

1.3. Mesenteric Venous Thrombosis (MVT)

Although MVT is considered among the etiologies of AMI, pathophysiology of venous obstruction significantly differs from arterial mesenteric ischemia. MVT may show a wide spectrum of clinical symptoms from a completely asymptomatic patient who is diagnosed incidentally to an acute, serious and life threatening disease. Predisposing factors for MVT include hypercoagulability, portal vein thrombosis, portal hypertension, a history of abdominal trauma or surgery and inflammation (Lang et al., 2014).

1.4. Non-occlusive Mesenteric Ischemia (NOMI)

NOMI is resulted from a seriously diminished blood flow to the intestine produced by a systemic low flow syndrome with secondary mesenteric vasoconstriction. Heart failure, hypovolemia and hypotension may cause these changes in mesenteric hemodynamics. Secondary vasoconstriction may be developed by the use of vasoconstrictive drugs such as vasopressin, catecholamines, β-blockers and digoxin (Bourcier, Klug and Nguyen, 2021).

2. Risk Factors

The risk factors that have been most commonly associated with AMI include systemic hypertension, atherosclerosis, atrial fibrillation, heart disease, digitais use, smoking and obesity. Considering causes of AMI, the risk factors of developing MAT include atherosclerosis, hypertension, dehydration, diabetes, dyslipidemia, antiphospholipid syndrome and estrogen therapy. The risk factors of MAE include recent myocardial infarction, congestive heart failure, atrial fibrillation, cardiomyopathies and embolisms due to atherosclerosis or aortic injury. The risk factors causing MVT include pregnancy, oral contraceptives, hypercoagulability syndromes, neoplasms, infectious causes, cirrhosis, portal hypertension, inflammatory diseases, right heart failure and chronic renal disease. The risk factors of developing NOMI include heart disorders, dialysis, shock, postoperative stress, burns, diabetes, pancreatitis, arrhythmias, hypovolemia, and the use of catecholamines, digitalis drugs and diuretics.

3. Pathophysiology and Epidemiology

The pathophysiology of AMI is mainly the result of insufficient blood flow to meet metabolic demand of the intestines (Oldenburg et al., 2004). Acute ischemia causes anaerobic metabolism in the gut, initial

hyperperistalsis and regional acidosis followed by severe ischemic pain from hyperperfusion of the intestinal wall (Kerzmann et al., 2018). This ischemic pain is projected across the abdominal wall where there is surface structure innervation (Lendzion et al., 2022). Thus, foregut structures produce visceral pain in the midgut, epigastrium, infraumbilical region and suprapubic area of the abdomen.

The intestines can tolerate significant reductions in the blood flow (Sise, 2014). At resting, about 80% of capillaries are not perfused without adequate delivery of oxygen. During hypoperfusion, the intestinal mucosa extracts an increasing amount of oxygen, preserving mucosal integrity at early stages of ischemia. Whereas, prolonged ischemia leads to an inflammatory reaction that impairs the mucosal barrier of the bowel, enabling translocation of bacteria. Once transmural necrosis has developed, systemic and local peritoneal inflammatory sequelae occur (Rosero et al., 2014).

Ischemia visceral pain is constant and intense and does not increase with palpation. This pain is not associated with peritoneal signs and abdominal wall rigidity on physical examination during the initial stages (Sise, 2014). This situation is described as the classic pain out of proportion to physical findings and is attributed to AMI. In later stages in which peritoneal inflammation develops, pain can be localized in the abdomen and associated physical findings can be observed.

3.1. Mesenteric Artery Thrombosis (MAT)

MAT usually occurs as a result of a ruptured atherosclerotic plaque in the proximal half of the mesenteric artery. MAT involves thrombosis of SMA, accounting for about 25% of cases, and is associated with previously existing chronic atherosclerotic disease resulting in stenosis in about 75% of these patients (Falkensammer and Oldenburg, 2006). In many of these patients, there is a history compatible with weight loss, “food fear” and postprandial pain. Receiving a detailed medical history is of paramount importance when evaluating patients suspected to have AMI. Thrombosis usually develops from the origin of visceral arteries. In the SMA an underlying plaque generally progresses to a critical stenosis leading to collateral beds. The nature of this condition is chronic and due to the development of collaterals patients can tolerate major visceral artery occlusion before the symptoms of MAT occurs. Symptomatic thrombosis of SMA is most often associated with celiac occlusion (Kärkkäinen and Acosta, 2017). Thrombosis of SMA may also occur because of mesenteric dissection, mycotic aneurysm or vasculitis. An

involved ileocolic artery will lead to necrosis of the proximal colon (Bala et al., 2022).

3.2. Mesenteric Artery Embolism (MAE)

MAE often occurs in the mid to distal end of the SMA and usually originates from the heart (Safioleas et al., 2008). Nearly half of AMI cases occur due to the embolism of SMA. Mesenteric emboli can originate from cardiac valves as in endocarditis, left ventricle such as left ventricular dysfunction associated with poor ejection fraction or left atrium (e.g. atrial fibrillation) (Bala et al., 2022). Emboli occasionally arise from an atherosclerotic aorta. Emboli are typically located at points of anatomic arterial narrowing. The SMA is especially vulnerable to MAE due to its larger diameter and take-off angle from the aorta. Most emboli are located 3-10 cm distal to the SMA origin and thus, preserve the colon and proximal jejunum. More than 20% of patients with MAE emboli are associated with concurrent emboli to other arterial beds such as the kidney and spleen (Acosta et al., 2005).

3.3. Mesenteric Venous Thrombosis (MVT)

MVT causes interstitial swelling in the intestine with subsequent disturbance of arterial flow, leading to the occurrence of necrosis (Abboud et al., 2008). MVT cases account for <10% of all AMI cases. In MVT, thrombosis is attributed to hypercoagulability, stagnant blood flow and endothelial damage that are known as “Virchow’s triad” (Bala et al., 2022). MVT occurs in 36% of young patients without a certain cause (Aschof et al., 2009). Acute pancreatitis or inflammatory bowel disease (IBD) leads to an inflammation process around the superior mesenteric vein (SMV), causing thrombosis. Surgical trauma such as bariatric surgery or splenectomy may also induce thrombosis. Hypercoagulability may develop because of inherited diseases including prothrombin mutation, protein C deficiency, protein S deficiency, antiphospholipid syndrome, Factor V Leiden and antithrombin deficiency. In addition, recent studies have shown that resistance to tissue plasminogen activator (tPA) is a risk factor for developing hypercoagulability (Moore et al, 2015). Oral contraceptives, hematologic disorders and malignancies may also cause thrombophilia (Cohn, Roshani and Middeldorp, 2007).

3.4. Non-occlusive Mesenteric Ischemia (NOMI)

Diffuse vasospasm of the visceral arteries including mesenteric arteries develops from sustained hypoperfusion to the region (Imanaka, Kyo and Abe, 2006). In NOMI, since no vascular occlusion is seen, pulsatile blood flow can be observed in the large arteries. NOMI accounts for approximately 20% of AMI cases and it usually occurs as a result of the vasoconstriction of SMA associated with decreased splanchnic blood flow (Aschof et al., 2009). The proximal colon is also affected from the compromised blood flow through the SMA due to involvement of ileocolic artery. NOMI patients usually suffer from concurrent illness such as cardiac failure, which may be precipitated by sepsis. The use of vasoconstrictive agents and hypovolemia may also promote NOMI.

4. Clinical Presentation

Numerous signs and symptoms of AMI are common in other intraabdominal pathologic conditions including small bowel obstruction, acute cholecystitis, pancreatitis and acute diverticulitis. The major clinical symptom is severe abdominal pain associated with nausea/vomiting, abdominal distension, diarrhea and bloody stool (Cudnik et al., 2013). However, the presence of clinical symptoms may be more subtle in elderly patients and in these patients, the clinical presentation of AMI is based on the underlying pathologic abnormalities. Patients with MAE or MAT usually have an acute-onset of symptoms and a rapid worsening of their clinical conditions, while patients with MVT or NOMI have a more gradual onset and more prolonged clinical course.

Patients with MAT usually report weight loss, postprandial pain and nausea that are associated with chronic intestinal insufficiency (Oldenburg et al., 2004). MAT patients with a subacute onset are prone to seek medical care later compared to those with MAE. However, when ischemia from MAT develops, patients present similarly to patients with MAE.

In MAE, symptom onset is typically dramatic due to lack of collateral circulation. MAE manifests as severe abdominal pain, nausea/vomiting and urgent bowel evacuation (Patterson, Kashyap and Dominique, 2022). The severity of abdominal pain is typically out of proportion to the physical examination. Excessive fluid loss and dehydration lead to tachypnea, tachycardia, circulatory collapse and mental confusion.

Patients with MVT usually present 1-2 weeks after symptom onset with the complaints of diffuse abdominal pain associated with diarrhea and anorexia (Oldenburg et al., 2004). The pain is most often localized in the lower quadrants. MVT causes less prodromal syndrome with postprandial pain compared to MAT. MVT may be resulted from surgery to the upper gastrointestinal tract, and inflammatory situations including thrombophilia and pancreatitis (Al-Diery et al., 2019). MVT from acquired hypercoagulability may develop in conjunction with oral contraceptives, intraabdominal inflammation or abdominal or multisystem trauma (Sise, 2014). The most common findings include hemoccult positive stool samples and abdominal distension. Large fluid losses and bloody ascites may occur, causing hypotension and dehydration, leading to worsening of AMI.

NOMI most commonly develops in critically ill elderly patients and those with serious mesenteric atherosclerosis with an acute hemodynamic insult (Amini and Nagalli, 2022). These patients are frequently sedated and intubated and thus, are unable to report their symptoms to the clinician. In this situation, AMI may not become clinically evident until hours or days after the insult. This is especially crucial in patients with severe hypotension treated using α-adrenergic agonists. These patients are prone to develop NOMI (Bourcier et al., 2021). NOMI patients often experience unexplaining worsening of their condition.

5. Diagnosis

AMI is difficult to diagnose because similar signs and symptoms are observed in several other conditions. Prompt diagnosis and treatment are of paramount importance, because AMI may proceed to fatal intestinal infarction. A compatible history, high index of suspicion and physical examination are mainstays of early diagnosis (Oldenburg et al., 2004). Once AMI is suspected, the diagnosis should be confirmed and appropriate treatment should be initiated timely. Severe abdominal pain is usually present, but other symptoms such as diarrhea, nausea and vomiting are not specific. AMI should be considered especially in the case of following conditions:

- ✓ A patient older than 60 years
- ✓ A history of atrial fibrillation
- ✓ Congestive heart failure
- ✓ Arterial emboli
- ✓ Recent myocardial infarction

- ✓ Postprandial abdominal pain
- ✓ Weight loss

5.1 Laboratory Tests

There is no sensitive specific test to diagnose AMI in early stages and laboratory parameters such as WBC, lactate and CRP can all be normal even if diagnosis of AMI is confirmed at laparotomy (Kassahun et al., 2008). Laboratory tests can be helpful in the diagnosis of AMI at late stages. Total leukocyte count >20000 may be useful with 80% sensitivity and 50% specificity. Metabolic acidosis and high D dimer are also useful (van den Heijkant et al., 2013). Studies have shown that low lactate concentrations can help to exclude the diagnosis of AMI, avoiding unnecessary laparotomies, particularly in older patients. Enzymes including alkaline phosphatase, creatinine kinase and lactate dehydrogenase may be helpful in the diagnosis of transmural infarction, but their sensitivity is low at the early stage of AMI (Ravipati et al., 2011).

The most common abnormal parameters in laboratory tests include leukocytosis, hemoconcentration and metabolic acidosis with high lactate and anion gap. High levels of serum aspartate aminotransferase, lactate dehydrogenase and creatine phosphokinase are frequently observed at the time of admission, but none of them have enough sensitivity to establish the diagnosis of AMI. Hyperkalemia and hyperphosphatemia are in general late signs and are often associated with bowel infarction (Franca et al., 2022).

5.2. Imaging Studies

Plain abdominal X-ray is not sensitive or specific sufficiently to help in the diagnosis of AMI (Chien-Hua et al., 2007). A simple X-ray is normal in up to 25% and shows nonspecific findings in 50% of AMI patients. It may reveal specific findings in the remaining 25% of these patients (Motta-Ramírez et al., 2013).

Ultrasound has a limited role in the assessment of AMI, because air distention and dilated bowel loops make this modality difficult or even impossible. It has been reported that Doppler ultrasound can show the occlusions in the SMA or celiac trunk with a sensitivity of 92-100% and a specificity of 70-89% (38). However, Doppler is not a recommended method in patients with a high index of suspicion of AMI (Kassahun et al., 2008).

Magnetic resonance angiography with gadolinium has been proved to have high sensitivity and specificity for visualization of stenosis or obstruction of the SMA (Shih and Hagspiel, 2007). The advantage of this method is not using ionising radiation. Its disadvantages include longer examination time, inability to visualize vascular calcium, lower spatial resolution and ineffectiveness in both non-occlusive and occlusion of distal branches (Oliva et al., 2013).

Computerized tomography (CT) is widely and quickly available and is accepted as the initial test of choice (Furukawa et al., 2009). Its sensitivity and specificity has been reported as 64% and 92%, respectively (Garzelli et al., 2020). CT is helpful in detection of signs of occlusion and ischemic vessel stenosis. The introduction of multidetector helical CT (MDCT) technique has improved the performance of CT dramatically by enabling rapid volumetric data acquisition for increased resolution, resulting in better identification of the cause, site and level of AMI (Schofield et al., 2014). With this development, sensitivity of MDCT has been reported as 90-100% and specificity as 100% (Kanasaki et al., 2018). MDCT angiography is now recommended as an accurate tool for fast diagnostic studies in patients with suspected AMI (Navas-Campo et al., 2020).

6. Treatment

Once the diagnosis of AMI is established, fluid resuscitation should be initiated promptly to enhance visceral perfusion. Nasogastric decompression should be started and electrolyte abnormalities should be corrected. Early hemodynamic monitoring should be implemented in order to guide effective resuscitation (Wyers, 2010). Since the risk of infection is high in patients with AMI, broad-spectrum antibiotics should be administered at an early stage of the treatment (Sartelli et al., 2021). Antibiotic therapy should be given for at least four days.

The goal of surgical intervention for AMI involves the establishment of blood supply to the ischemic bowel, preservation of viable bowel and resection of necrotic regions. Intestinal viability is the most critical factor affecting survival in patients with AMI.

6.1. Laparotomy

After the initial fluid resuscitation, midline laparotomy is performed followed by the evaluation of all intestinal areas with a decision to resect all necrotic parts. The SMA can be palpated by placing fingers behind the root of

the mesentery. For proper exposure aiming revascularization, direct sharp dissection is performed to expose the artery from the surrounding mesenteric tissue. Arteriogram is the study of choice in case of diagnostic uncertainty. Arteriogram can be carried out intraoperatively. Intraoperative duplex is rapid, easy to perform, repeatable and frequently definitive alternative diagnostic technique (Bala et al., 2022).

6.2. Revascularization of the ischemic bowel

In the multidisciplinary approach to AMI, revascularization of the blood supply plays a crucial role. In a study by Chou et al. with 104 AMI patients, 62% of the patients who did not undergo revascularization died, while this rate was 42% in the patients who were re-vascularized (Chou et al., 2021). Revascularization technique depends on the pathophysiology of AMI.

In MAE patients, embolectomy and angioplasty are well-established treatment modalities. In MAT patients who had thrombosis at the origin of the aorta a bypass procedure will be needed. Bypass procedure can be carried out in either retrograde approach from the common iliac arteries or infrarenal aorta or an antegrade approach from the supraceliac aorta. Revascularization of SMA alone is usually sufficient in acute settings. Today endovascular procedures reduce the need for bypass. SMA shunting should be considered in the case where necessary technical skills are not available. NOMI and MVT do not require vascular repair. Before the surgical procedure, full-dose anticoagulation should be started in all patients (Bala et al., 2021).

6.3. Laparoscopy

As a bedside procedure, diagnostic laparoscopy is an appropriate approach in the intensive care unit (ICU). Its advantages include preventing adverse effects during transfer of the critically ill patients and diminishing time delay for awaiting availability of the operating room. However, diagnostic laparoscopy is not a routinely adopted procedure (McGinty, Hogle and Fowler, 2003).

Second-look laparoscopy may be a helpful alternative to conventional surgery. This technique can be performed as an ICU bedside operation and prevents patients from the risks of relaparotomy and trauma. Laparoscopy can be helpful in confirming the diagnosis, and evaluation of the extent of the ischemic small intestine segment. It provides a treatment option in the case of segmental necrosis.

6.4. Endovascular Procedures

Recently, endovascular techniques have become popular in re-establishment of the blood supply to the SMA. Since AMI patients are physiologically different and heterogenic, there is no randomized controlled study to assess and compare open surgery and endovascular approach (Orr and Endean, 2015). Primary management of AMI using endovascular procedures is a controversial issue (Smith et al., 2013). Some studies have reported significantly lower rates of mortality, less bowel resection and lesser need for laparotomy with endovascular approach (Zhang et al., 2017). Open surgery is more effective in evaluating the viability of the intestine and thus prevents delays in revascularization, particularly when an endovascular technique is not available.

CONCLUSION

AMI is a serious surgical emergency. Frequently associated concomitant health problems and delays in the diagnosis and treatment have made AMI a highly morbid and often fatal disease. Therefore, early diagnosis and treatment are the mainstay in the management of AMI. For all survivors of the various forms of AMI, lifelong treatment of their comorbidities is essential. The diagnosis and treatment depend on the underlying pathology. Efficient patient management relies on a multi-disciplinary approach in the treatment of AMI. The most important prognostic factor that can be affected by the clinician is the time elapsing between symptom onset and definitive treatment.

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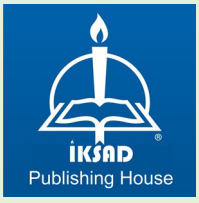

ISBN: 978-625-6404-99-1