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CONTENTS

CHAPTER 1

DENTAL ANXIETY IN CHILDREN AND MEASUREMENT METHODS

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Dental anxiety is described as the apprehension and fear of any dental procedure, irrespective of any external factors, and is most often manifested in childhood or adolescence. (Folayan et al.,2004; Smyth,1993). This condition can greatly affect the daily life of children and can become permanent in adult life if left untreated (Bersntein et al.,1996; Ost, 1987). In the development of anxiety, direct factors such as negative dental experiences in previous appointments, as well as indirect experiences heard from family members and individuals in the immediate environment have a great influence (Berggren et al.,1997; Bayrak et al.,2010)

Dental anxiety and fear is considered an important public health problem in many countries (Stabholz & Peretz, 1994; Carillo-Diaz et al., 2012). If this is not managed by dentists with appropriate behavioral guidance techniques, it can lead to fear of visiting the dentist in children. It may also cause children to postpone dental appointments and abandon oral hygiene practices and habits(Carillo-Diaz et al., 2012; Folayan et al., 2002; Peretz & Zadik, 1994).). In addition, people with high dental anxiety visit dental offices only in emergencies or when they experience a toothache, which prevents primary care, increases the number of decayed teeth, worsens oral hygiene, makes treatment difficult, and increases dental costs. (Peretz & Kharouba, 2013). The ability of dentists to perform all dental treatments effectively and efficiently and to create positive behavioral attitudes toward pediatric patients is hindered by the anxiety that children show during dental treatments (Rayen et al., 2006; Wright et al., 2014). Therefore, it is very critical for dentists to be able to evaluate anxiety levels in children who need special attention and support (Rayen et al., 2006).

Dental Anxiety and Fear

Anxiety and fear are defense mechanisms that people show against all kinds of dangers throughout their lives (Şimşek & İspir, 2019). Fear is a vital defense mechanism against a tangible threat, whose source is obvious and external, which can be clearly expressed and which is not fundamentally affected by internal conflicts (Başoğul & Buldukoğlu, 2015). Unlike fear, anxiety, on the other hand, is a defense mechanism against an intangible threat of unknown origin and originating from internal conflicts. In anxiety, the trigger of escape behavior is physiological stimuli and it is a subjective state of tension against the perceived danger (Başoğul & Buldukoğlu, 2015; Kronina et al., 2017).

Dental anxiety is a specialized version of general anxiety and is considered to be a sense of loss of control associated with the feeling that something very bad is going to happen and a state of apprehension about dental procedures. Dental fear, on the other hand, is recognized as a normal emotional response to known and threatening effects during dental treat-

ments (Shindova & Belcheva, 2021).

Although dental fear and anxiety can be seen at any age, it usually begins to develop in childhood or adolescence. Dental anxiety and fear, which begin in childhood, continue later in life and cause a cycle that results in the avoidance of dental treatments, resulting in a negative impact on oral and dental health. Therefore, it is of great importance for dentists to detect dental anxiety in childhood (Buchanan & Niyen, 2002; Skaret et al., 1998).

Etiology of Dental Anxiety

Behavior management or dental anxiety in pediatric patients is associated with many factors of internal or external origin. The factors that cause dental anxiety in children can be examined in three main categories: individual, dental and environmental factors (Paulsen, 2009).

Individual Factors

Age is one of the biggest factors in the formation of dental anxiety and behavioral problems during treatment (Paulsen, 2009). The psychological development of children differs with age. This difference affects the child's understanding and adaptation processes to dental treatments. Since the psychological development of young children may not be at a level to understand and tolerate dental treatments, age is an essential consideration in cooperation and dental anxiety problems (Klingberg & Broberg, 2007).

One of the factors affecting the occurrence of dental anxiety is the sex of the child. In many studies investigating the effect of sex on dental anxiety, was reported that the level of dental anxiety is higher in females than in males (Boka et al., 2017; Chhabra et al., 2012; Lee et al., 2007; Peretz & Efrat, 2000). The higher level of dental anxiety in females compared to males is explained by the fact that females have lower pain tolerance than males and male patients show their fears less due to their upbringing (Yetiş & Küçükeşmen, 2013). It is also thought that the different structural and functional characteristics of the brain in men and women cause dental anxiety to be different between the sexes (Schienle et al., 2013).

Another factor affecting dental fear and anxiety is the personality traits and temperament of the child. It has been reported that dental anxiety is associated with personality traits and is more common in people with negative moods, impatient, shy, aggressive, easily irritable when disappointed, and prone to violence in bilateral relationships (Arnrup et al., 2002; Locker et al.,2001).

Studies indicate that children with neuropsychiatric problems such as attention deficit hyperactivity disorder, autism spectrum disorder, depres-

sion and anxiety disorders, Tourette syndrome, Asperger syndrome, and psychological-mental developmental retardation, which are estimated to occur in approximately 5% of children, show more dental anxiety and behavioral problems (Gustafsson et al., 2010).

Environmental Factors

Social learning theory suggests that dental anxiety is developed by observing and imitating the anxious behaviors of others. Although it is mostly mothers or carers from whom children learn about anxiety, children also learn about dental anxiety from their siblings, relatives, and friends through social learning. (Yetiş & Küçükeşmen, 2013). It has been argued that anxiety acquired from the family is related not only to the anxiety level of the parents but also to the educational level, socioeconomic and cultural status of the parents (Önçağ et al., 2005; Stabholz & Peretz, 1994). It has been reported that children of families with high socioeconomic status show better compliance during dental procedures, dental fear, and anxiety levels are more common in societies with low socioeconomic standards, and the quality of life-related to oral health of children of parents with high dental anxiety levels is lower. (Klingberg, 1995; Neverlien, 1990; Nicolas et al., 2010).

The behavioral development of the child and his/her reactions to different situations are determined by the behavioral patterns of the parents and the parent-child relationship (Aminabadi et al., 2012). The attitudes and behaviors of the family have an essential role in the child's reactions to an unfamiliar environment and situation, such as the first visit to the dentist. Children raised in different family types have different positive or negative outcomes (Aminabadi et al., 2015; Bailey et al., 1973).

Dental Factors

One of the biggest factors determining the reaction of the child to dental treatment is the attitude and behavior of the dentist. The first visit to the dentist is an important factor in how the child will behave against the environment and conditions in subsequent appointments. Positive communication between the dentist and the patient increases the patient's satisfaction and cooperation and increases the child's habit of going to regular dental appointments and motivation toward oral hygiene practices (Lee et al., 2007). The age, sex, and appearance of the dentist who will perform the treatment can also affect patients' preferences in choosing a dentist (Brosky et al., 2003).

Children's previous dental experiences, especially painful dental treatments, have an essential role in the formation of dental anxiety or fear

(De Jongh et al., 2005). Poor dental treatments are one of the main factors in the formation of negative dental experiences by affecting subsequent dental visits due to the child's conditioning (Davey, 1989; De Jongh et al., 2005; Liddell & Locker, 2000; Locker et al., 1996). It is also known that there are individuals who do not have dental anxiety despite having had traumatic dental experiences before. Based on this, some researchers argue that dental anxiety is not caused by bad dental experiences in the past, but by individual perceptions such as unpredictability, anxiety about the unknown, and discomfort about not being in control (Armfield, 2010).

Dental Anxiety Measurement Methods

Currently, subjective and objective measurement methods, including behavioral scoring, psychometric measurements, and projective and physiological measurements, are used to measure dental anxiety (Bayrak et al., 2010). In children, the selection of the appropriate method depends on the age and mental maturity of the child (Guinot et al., 2011).

Behavior Scoring

The behavioral scoring method is a simple visual assessment of behavior that is frequently used to determine dental anxiety. Although the child is not necessary to respond to questions about dental anxiety, it is usually based on a descriptive scale or assessment of facial expressions and observation of the child's behavior during dental treatment based on visual scoring. (Bayrak et al., 2010; Klein et al., 2015; Nelson et al., 2016; Yahyaoğlu et al., 2017). According to this purpose, the Frankl Behavioral Scale (FBS), Modified Yale Preoperative Anxiety Scale (mYPAS), Clinical Anxiety Rating Scale/Uncooperative Behavior Rating Scale (CARS/BRS), Observation-based measurement methods such as Behavior Evaluation Scale (BES) and Behavior Profile Rating Scale (BPRS) have been developed (Aartman et al., 1996; Yahyaoğlu et al., 2017).

Frankl Behavioral Scale

It is a scale that classifies children's behaviors during dental treatments into four categories according to the child's attitude and level of cooperation. It is frequently used in dental practice and research due to its ease of learning and use and quick and easy classification (Aapd, 2015). Behavior scores are classified and scored in four categories ranging from 1 to 4: strongly positive (4), positive (3), negative (2), and strongly negative (1) (Yıldırım et al., 2016). The score definitions of the Frankl behavior scale are shown in Table 1.

Table 1. *Frankl behavioral scale (Yıldırım et al., 2016)*

Rating	Attitude	Definition
1 (--)	Definitely Negative	Symptoms of severe negative behavior, such as opposition to treatment, constant crying, excessive fear
2 (-)	Negative	Signs of negative behavior: reluctant to accept treatment, sullen, withdrawn
3 (+)	Positive	Accepting, curious, compliant, shy, communicative but cautious approach to treatment
4(++)	Definitely Positive	Engaged in treatment, happy with the environment, cooperative, and smiling

Modified Yale Preoperative Anxiety Scale (Mypas)

Created in 1997 by modifying the Yale Preoperative Anxiety Scale, it is a scale applied to patients older than 2 years in many branches of health care. The Modified Yale Preoperative Anxiety Scale has 22 items categorised into 5 domains: level of activity, emotion, speech, degree of reaction and communication with parents. The highest level of behavior in each category determines the score of that category. When scoring the scale, the scores marked by the patient in each category are summed and divided by the maximum score that can be obtained from that category. For 5 different categories, the same procedure is applied and all scores are summed. The summed value is split by the total number of categories, i.e. 5, and the total value is multiplied by 100 to get a score between 23.33 and 100. In the results obtained, higher values indicate higher anxiety levels (Jenkins et al., 2014; Kain et al., 1997;).

Physiological Techniques

As a result of dental anxiety, some changes such as hyperventilation in the respiratory system changes in blood pressure and pulse speed in the cardiovascular system, increased muscle tone in the muscles, constipation, stomach spasms, diarrhea, and dry mouth due to salivary glands are observed in the digestive system. (Guinot et al., 2011). Due to these physiological changes, many studies have reported that heart rate, blood pressure, respiratory rate, salivary cortisol, and sweat test measurements are safe

parameters for measuring anxiety (Rayen et al., 2006). For this purpose, special equipment such as a sphygmomanometer and pulse oximetry are used. However, since the equipment used in this method can also induce an anxiety reaction in the child, it can cause dental anxiety even in a carefree child. Since it is not possible to attribute the results to dental anxiety alone in physiologic measurement methods, inaccurate and non-specific results may also occur. For all these reasons, physiologic measurements have limited clinical application in the routine measurement of dental anxiety (Aartman et al., 1996; Bayrak et al., 2010).

Projective Techniques

Projective techniques aim to assess the level of fear and anxiety by narrating purposefully drawn pictures by children or by drawing objects or creatures that may be the object of fear in the child. Projective tests are more preferred in young children because of their inadequate mental development and poorer comprehension (Bayrak et al., 2010; Eichenbaum & Dunn, 1971). In projective techniques, it is aimed at determining the emotion they feel by showing pictures or figures to children. The Facial Image Scale, Venham Picture Test (VPT), Children's Dental Fear Picture Test (CDFP), Human Figure Drawings (HFD), Visual Analog Scale (VIS), and Child Drawing: Hospital CHCS are among the projective techniques (Aartman et al., 1998; Buchanan & Niven, 2002; Venham & Gaulin-Kremer, 1979).

Venham Picture Test (VPT)

It is a scale consisting of a total of 8 different cards, each card containing two different figures, one anxious and one non-anxious (Figure 1). While 1 point is given to the pictures of anxious children, 0 point is given to the pictures of non-anxious children. Children are asked to point to the figure that most reflects them at that moment and scoring is done between 0-8. A point 0 means that there is no anxiety, while a point 8 means that there is high anxiety. (Buchanan & Niven, 2002; Venham & Gaulin-Kremer, 1979).

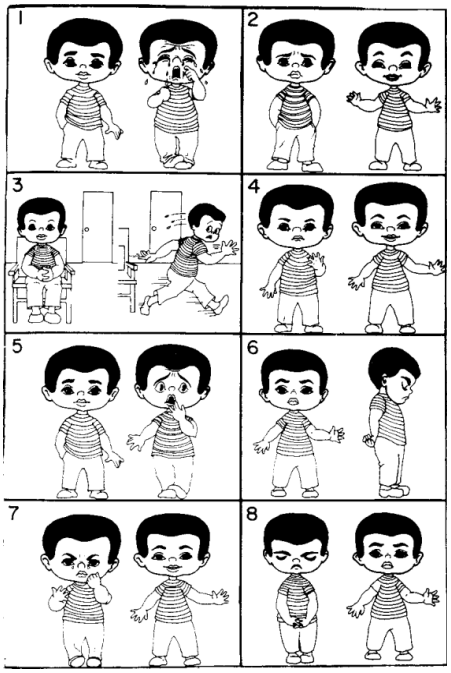

Figure 1: Venham picture test (Buchanan & Niven, 2002)

The Facial Image Scale (FIS)

It is a situation scale consisting of five facial expressions ranging from very happy (1 point) to very unhappy (5 points), developed to ensure quick results from young children (Figure 2). Children are asked to choose the facial expression that they associate with themselves according to their current emotional state. In the scoring of the scale, the happiest facial expression receives a score of 1, while the unhappiest facial expression receives a score of 5. The higher the points, the higher the children's anxiety scores. (Shindova & Belchava, 2021).

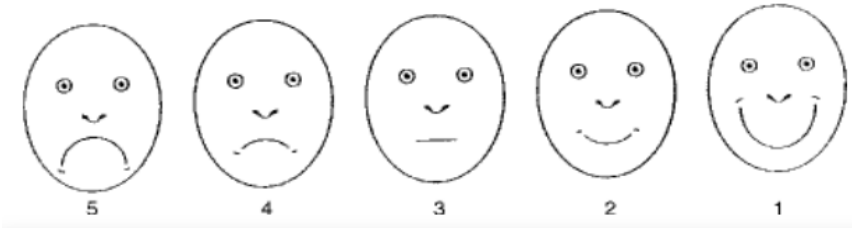

Figure 2: Facial expression scale (Buchanan & Niven, 2002)

Smiley Faces Program (SFP: Smiley Faces Program)

It is a computerized version of the Corah Dental Anxiety Scale and consists of 4 questions about experiences that children may have had before. Dental anxiety is determined by selecting facial expressions in the answers. The advantages of this program are that the test can be done in a short time, the data obtained can be stored, and can be easily standardized. In addition, the shapes on the computer increase children's interest in the questions and make it more fun. It has been reported that it can be used in children between the ages of 4-11 years (Buchanan, 2010).

Psychometric Measurements

Psychometric scales are questionnaire-type scales that also assess specific situations related to the dental environment or practice. These question-and-answer scales allow the child to express themselves verbally. Depending on the age of the patient, they can also be administered to younger children by having their parents or caregivers answer the questions on their behalf. Psychometric tests are widely used because of their ease of administration. In addition, it is a subjective evaluation method that facilitates statistical study with the numerical values obtained as a result of the scale (Bayrak et al., 2010; Guinot et al., 2011; Porrit et al., 2013). Corah Dental Anxiety Scale (Corah Dental Anxiety Scale, DAS), The Dental Subscale of the Children's Fear Survey Schedule (CFSS - DS), Modified Child Dental Anxiety Scale (MCDAS), Abeer Child Dental Anxiety Scale (Abeer Child Dental Anxiety Scale, ACDAS), Spielberger's State-Trait Anxiety Inventory For Children (STAIC), Smiley Faces Program (SFP), The Index of Dental Anxiety and Fear (IDAF-4C+) are among the psychometric measures (Buldur & Armfield, 2018).

Corah Dental Anxiety Scale (DAS)

The scale was developed to evaluate dental anxiety in adults and consists of four questions on a scale of 1 to 5. Participants indicate how comfortable or anxious they feel about four situations related to dental treatments by choosing from a series of responses. When the points for each item are summed, a score interval between 4 (not anxious) and 20 (extremely anxious) is obtained (extremely anxious) (Corah et al., 1978). Although this scale is usually used with adults, it can also be used with children by having the questions read by the parent or by the child if the child can understand the questions (Buchanan & Niven, 2002).

Modified Child Dental Anxiety Scale (MCDAS)

The Corah dental anxiety scale was developed with modifications to make it more comprehensible to children due to its complexity (Wong et

al.,1998). The scale includes 8 questions related to dental treatments such as tooth extraction, local and general anesthesia, and scaling. Each question has 5 response optionsResponse scales vary from a minimum of 8 points to a maximum of 40 points. There are some disadvantages to this scale, such as not being able to understand the questions asked about dental treatments (Goettems et al., 2012). To facilitate its use in young children, the Modified Children's Dental Anxiety Scale-Faces Adaptation (MCDAS-f) was developed by adding five different facial expressions matched with numbers to the original form (Howard & Freeman, 2007).

The Dental Subscale Of The Children's Fear Survey Schedule (CFSS - DS)

The scale used to assess children's dental anxiety was developed from the Child Fear Questionnaire Schedule, which is designed to assess various fears and anxieties of children and consists of a total of 80 items. The CFSS-DS is a dentistry-specific measure consisting of 15 questions about dental treatment and dental equipment that allow children to assess how afraid they are of dental situations or treatments. The answers to the questions were graded according to five response scores between "not afraid at all (1)" and "extremely afraid (5)".According to the score obtained, those with a score between 0-38 are considered to have no anxiety, while those with a score of 45 and above are considered to have high anxiety (Buldur & Armfield, 2018; Guinot et al., 2011; Porritt et al., 2013). The disadvantage of the scale is that it cannot assess physical reactions, thoughts, and behaviors that may contribute to measuring dental anxiety in children (Porritt et al., 2013).

Abeer Child Dental Anxiety Scale (ACDAS)

Developed in 2010, it is a 19-question scale with 3 sections: dental, cognitive, and child assessment. In the dental section, 13 questions about the experience of visiting the dentist are asked and the answers are scored with happy (1 point), normal (2 points), and frightened (3 points) facial expressions. The resulting score ranges from a low of 13 points to a high of 39 points, with scores higher than 26 indicating high dental anxiety. The remaining cognitive and child assessment sections of the scale are completed by the child's parent or legal guardian and the dentist. This scale is a valid cognitive scale for the evaluation of dental anxiety in children over 6 years of age. (Aslan et al., 2021).

The Index Of Dental Anxiety And Fear, (IDAF-4C+)

This index is a modular scale built on sound theoretical and psychological foundations. It is designed to effectively address the limitations of

existing dental fear and anxiety scales [45]. Although the IDAF-4C was originally designed for adult patients, it has shown good reliability and validity in children in several studies (Buldur and Armfield, 2018; Carillo-Diaz et al., 2013).

The scale consists of three modules (IDAF-4C, IDAF-P, IDAF-S). The IDAF-P is used to detect the presence of dental phobia but is also useful for studying the etiology and management strategies of dental phobia. The phobia module includes five items about phobic dispositions. The answer format is yes or no. The IDAF-S is useful to collect more data about the participant's fear of certain dental stimuli (needles, tooth extraction, pain). The stimulus module contains ten statements with response choices ranging from one (not at all) to five (a lot). The items identify potential causes of anxiety and worry during dental visits, such as painful or unpleasant treatments, feelings of embarrassment and shame, and the inability to control what is going on (Armfield, 2010). The phobia (IDAF-P) module is used for epidemiological studies, while the stimulus (IDAF-S) module is used for clinical purposes.

The IDAF-4C module is the main module that identifies the participant's overall degree of dental fear. It contains eight questions, each consisting of two items related to the emotional (I am afraid when I go to the dentist), behavioral (I put off making an appointment to go to the dentist), cognitive (I think about everything that goes wrong before I go to the dentist) and physiological (my heart beats faster when I go to the dentist) factors of dental anxiety and fear. The response format consists of five options that range from one (disagree) to five (strongly agree). The total score for the IDAF-4C module is computed as the mean score of the eight items (Armfield, 2010). The higher the average scale point, the higher the degree of anxiety. Mean full-scale scores are categorized as 'No or little dental fear' (score range 1-1.5), 'Low dental fear' (score range 1.51-2.5), 'Moderate dental fear' (score range 2.51-3.5), and 'High dental fear' (>3.5) (Armfield, 2010; Carillo-Diaz et al., 2012).

Spielberger's State-Trait Anxiety Inventory For Children (STAIC)

It is a scale designed by Spielberger et al. in the form of two separate scales of 20 questions in order to determine the momentary state of anxiety, i.e. "state", and the anxiety and unhappiness that the individual feels continuous, i.e. "trait". The answers given to the state anxiety scale were "never, a little, a lot, and completely", while the answers given to the trait anxiety scale were "never, sometimes, most of the time, always". ". This scale, which is frequently used in children, is generally preferred in the 8-12 age range. Although the reliability and validity of both scales are

high, their use is limited due to the length of the questions and the time it takes. For this reason, a shorter modification consisting of 6 questions was created (Guinot et al., 2011; Özusta, 1995; Vlad et al., 2020).

CONCLUSION

Dental anxiety is a condition that can be seen in every population and age group and can cause disruption of dental procedures. It can be very difficult to determine the presence and level of dental anxiety especially in pediatric patients. Measurement of anxiety levels in pediatric patients with existing dental anxiety assessment methods will be very useful for dentists during the treatment of pediatric patients. Depending on the age group of the children, it may be preferable to use several different methods together. Dentists should be aware of current and innovative methods and apply these methods effectively in identifying and coping with dental anxiety, which is seen in a large mass of society and encountered as an obstacle in dental treatments.

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CHAPTER 2

THE ROLE OF NUTRITION AND RESISTANCE EXERCISE IN SARCOPENI

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INTRODUCTION

The aging population in our country, as well as the rest of the world, has grown over time. According to the Turkish Statistical Institute, the senior population in Turkey has climbed by 22.5% in the last five years, reaching 9.5% of the population, and is expected to reach 22.6% by 2060 (Zeybek et al., 2022). The increase in life expectancy and the decline in birth rates result in an increase in the elderly population, both numerically and in comparison to the general population (Kaya et al., 2020). As the aged population grows, so do the issues of old age. Every ten years, the old population drops while fragility and commitment rise (Dos Santos et al., 2017; Moreland, 2020). Most chronic diseases, which are defined by gradual molecular, cellular, tissue, and organic functional degradation over time are associated with increased chronological age (Boengler et al., 2017). Sarcopenia is most common in the fifth decade of life, affecting 9.9% to 40.4% of older persons (Cruz-Jentoft et al., 2010; de Sire et al., 2022). Sarcopenia is regarded as a notable feature that accumulates over a lifetime among the aged, yet it indicates a muscle disease (muscle failure) rooted in deleterious muscle alterations that may develop in progeria (Brook et al., 2016). Despite this, the European Working Group Sarcopenia (EWGSOP) published progress and updates of the definition of sarcopenia in the age and aging, aiming to achieve a consensus definition of it. Sarcopenia is gradually being used in other relevant systems aside from the skeletal muscle system, such as diabetes, cancer, and the endocrine system (Fukuoka et al., 2019; He et al., 2022).

Sarcopenia, defined as decreased skeletal muscle mass and function, is a geriatric syndrome that worsens with age (Cruz-Jentoft et al., 2019; Makizako, 2019). Age-related sarcopenia affects about 6-22% of older persons (Dent et al., 2018). Sarcopenia is most commonly found in the elderly, however it can also be detected in young individuals (Cruz-Jentoft et al., 2010). Sarcopenia has been found in studies to raise the chance of older people's health demands and hence health care costs (Sirven et al., 2017; Antunes et al., 2017). These loads are directly related to the economic burden, which accounts for a larger portion of the health-care system burden (Floransa et al., 2018; Voulgaridou et al., 2023). In a meta-analysis investigating the incidence of sarcopenia in older persons in residential nursing homes and hospitals in 2020, Papadopoulou et al. (2020) found that the prevalence of sarcopenia in the senior population living in the community ranged from 8% in women to 11% in men. Sarcopenia was found in 51% of men and 31% of women in nursing homes and hospitals, and the prevalence of sarcopenia increased unexpectedly. In our country, research on sarcopenia is minimal. The prevalence of sarcopenia was 19.5% in males and 13.6% in women among the 100 seniors who applied to Hacettepe

University to verify the reliability of muscle ultrasonography in the diagnosis of sarcopenia, and the prevalence of sarcopenia was 16% (Kuyumcu, 2014).

Global sarcopenia research has increased rapidly from 2001 to 2023. The contribution of the United States to this field is quite high (Yuan et al., 2022). The sarcopenia study will continue to focus primarily on nutrition and exercise, and its relationship with aging and other diseases, and molecular mechanisms are explored.

Pathogenesis of Sarcopenia

As putative molecular markers of sarcopenia, factors encompassing muscle-specific processes (e.g., mitochondrial dysfunction in skeletal myocytes) and systemic mediators belonging to multiple domains (e.g., inflammation and amino acid dysmetabolism) have been identified (Picca ve ark., 2018; Picca and Calvani, 2021). Sarcopenia is a multifactorial disorder characterized by muscle mass loss that can be caused by aging, eating habits, sedentary lifestyle, and other diseases (Kirwan et al., 2020; de oilivera Zanuso et al., 2022). While the original criteria centered on muscle mass loss, they are now based on muscular strength and function, as demonstrated by a number of international standards, rather than merely on muscle mass (Sayer and Cruz-Jentoft, 2022). The Asian Sarcopenia Study Group (Chen et al., 2020) describes Sarcopenia as "age-related loss of muscle mass, plus reduced muscle strength and/or poor physical performance". As a result, while sarcopenia is sometimes confused with cachexia and malnutrition, it differs based on the variables associated with reduced muscle mass. Sarcopenia is regarded as a notable feature that accumulates over a lifetime among the aged, yet it indicates a muscle disease (muscle failure) rooted in deleterious muscle alterations that may develop in progeria (Brook et al., 2016). Figure 1 depicts the distinctions between sarcopenic and cachectic muscle (Sayer and Cruz-Jentoft, 2022).

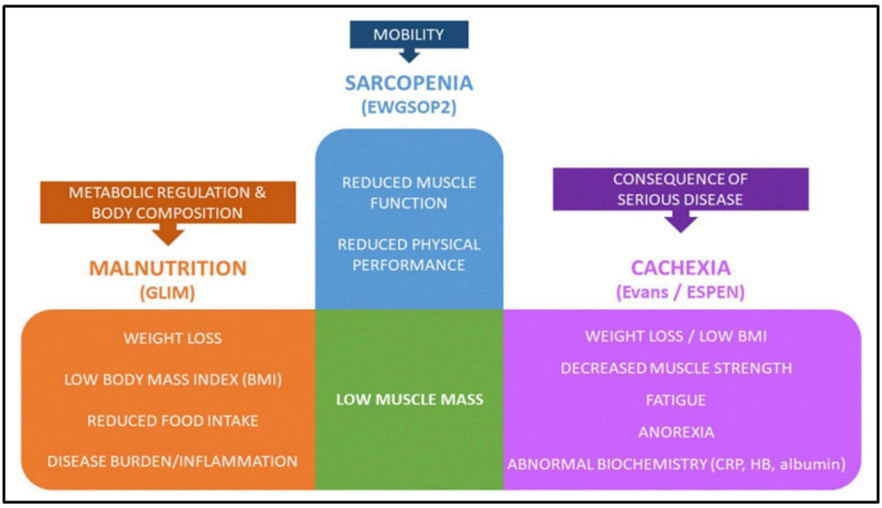

Figure 1. *Factors of sarcopenia affecting low muscle mass compared to cachexia and malnutrition*

Sarcopenia's pathogenesis is linked to an imbalance between muscle cell creation and muscle cell death. With the problems caused by age, the number of muscle cells continues to deplete with time, anabolic stimulation, decreased mitochondrial function and loss of insulin sensitivity, reduced neuromuscular signaling with changes in gene expression become resistant (Pár et al., 2021; Guo et al., 2023; Banack et al., 2022; Mellen et al., 2023). Individual variances in skeletal muscle number and quality exist, even when age, gender, degree and kind of physical activity, and macro- and micronutrient diets are included. Genetic factors account for this variation (Semenova et al., 2023).

Sarcopenia causes an increase in skeletal muscle protein breakdown and a decrease in protein synthesis (Fielding et al., 2011; Kakehi et al., 2022). At the age of 30, our body has the most muscle mass. After the age of 40, our muscle mass begins to decline, with the loss of muscular mass accelerating with age (Son, Yu and Seo, 2019; Çolak and Çiftçi, 2021). More attention has been paid to the identification of effectors that contribute to sarcopenia, such as impaired mitochondrial function, a decrease in the number of motor units, and a decrease in the number and regenerative capacity of muscle stem cells (MuSCs), all of which correlate with cellular senescence in skeletal muscle (Wiedmer et al., 2021).

Types of sarcopenia

When there are no other clear causes other than old age, primary sarcopenia is accepted. Secondary sarcopenia occurs when this condition develops as a result of other factors such as sedentary lifestyle, sickness, or

malnutrition (Cruz-Jentoft et al., 2010). Prolonged bed rest due to activity, sedentary life, astronauts' lifestyle, insufficient energy intake, protein quality and insufficiency, nutritional disorders and gastrointestinal problems, anorexigenic drugs, pathologies affecting absorption in intestinal function, such as abdominal hernias and inflammatory bowel disease, are all potential causes of secondary sarcopenia (De Andrade et al., 2015; Clark et al., 2020). When our body is exposed to an invasion or acute inflammation, an acute biological reaction begins to develop that affects neuroendocrine, immune mechanisms and metabolism. The insulin resistance effect of catecholamines and cytokines increases blood glucose levels, resulting in increased lipolysis with increased protein catabolism. Therefore, sarcopenia may be related to the prognostic processes of the diseases (Kakehi et al., 2022).

The distinction between primary and secondary sarcopenia is critical for therapy planning. Because the treatment of the underlying secondary causes of sarcopenia takes into account the prevention of excessive loss in the majority of disorders. We can protect our patients from mortality and morbidity by increasing muscle mass and performance and avoiding consequences from other disorders. Table 1 summarizes the major and secondary causes of sarcopenia (Cruz-Jentoft et al., 2010).

Table 1. *Arranged according to the recommendations of the European working group*

Primary sarcopenia	
Age-related sarcopenia	No other cause evident except ageing
Secondary sarcopenia	
Activity-related sarcopenia	Bed rest, sedentary lifestyle, deconditioning, or zero-gravity situations can all cause this.
Disease-related sarcopenia	Linked to progressive organ failure (heart, lung, liver, kidney, and brain), inflammatory illness, cancer, or endocrine disorders
Nutrition-related sarcopenia	Anorexia is caused by a lack of energy and/or protein in the diet, such as malabsorption, gastrointestinal diseases, or the use of anorexia-causing drugs.

Iatrogenic sarcopenia

Iatrogenic sarcopenia is a disorder that develops from activity-related conditions, primarily muscle atrophy caused by prolonged bed rest and

immobilization, and is currently noticed after hospitalization. Sarcopenia generated by the activity of healthcare personnel in a healthcare facility is referred to as iatrogenic sarcopenia (Nagano et al., 2019). Another condition that can cause iatrogenic sarcopenia is nutritional sarcopenia. It has a negative impact on the patient, causing dysphagia as a result of the prolonged treatment period and the use of swallowing-related muscles that atrophy in patients who temporarily stop taking in food due to aspiration pneumonia (Maeda et al., 2016; Kakehi et al., 2022). In acute care hospitals, the situation gets worse if dysphagia is not treated and dietary needs are not properly managed (Iwamoto et al., 2014). Pathophysiological issues, medical procedures, medical errors, and hospital infections can also cause iatrogenic sarcopenia, a different type of sarcopenia that can result from medical intervention (Nagano et al., 2019).

Reduced physical activity and dietary modifications, two of the disease's lifestyle-related impacts, raise the chance of chronic renal failure, one of the kinds of sarcopenia that results from the condition. According to reports, sarcopenia affects between 5-62.5% of people with chronic renal failure, and the risk rises as the disease progresses (Barreto Silva et al., 2022).

Tissue modified diets (TMDs) are given to older adults in aged care facilities who have dysphagia, inadequate dentition, or cognitive-behavioral eating problems to help them chew and swallow (Engh and Spever, 2022; Wu et al., 2022). Tissue modified diets (TMDs) have been observed to impact nutritional status in post-stroke patients, resulting in the consumption of lower food levels and associated with the incidence of sarcopenia. This is an example of sarcopenia produced by post-illness nutritional status (Shimizu et al., 2022).

Bioimpedance analysis for muscle mass, DEXA, CT, MRI, potassium/fat-free weight, muscle strength test, knee flexion/extension, and peak expiratory flow are used to diagnose sarcopenia. Tests for measuring physical performance include the short physical performance battery, walking speed, the get up and go test, and the power to climb stairs (Karpuzcu, 2020; Can and Şanlıer, 2022). Figure 2 shows the EWGSOP-2 protocol for sarcopenia diagnosis. Discover the situation, determine the diagnosis, and determine the severity. The EWSGOP2 (Cruz-Jentoft et al., 2010) criteria are a development of the EWGOP1 (Cruz-Jentoft et al., 2019) algorithm, which was proposed by the European Working Group on Sarcopenia (EWSGOP). EWGSOP2 also suggests using the chair stand test (Cesari et al., 2009) and grip strength (Dodds et al., 2014) to evaluate muscle strength. The Double-emission X-ray Absorptiometry (DXA) and Bioimpedentiometry Analyses (BIA) are recommended for measuring muscle mass and quality in standard clinical care, while the use of MRI or CT is only advised for

research or for specialized needs in patients who are at high risk of having unfavorable clinical outcomes (Romani et al., 2022). The artificial intelligence system will help in the evaluation of sarcopenia, including in clinical applications (Rozynek et al., 2021; Pekcan, 2022).

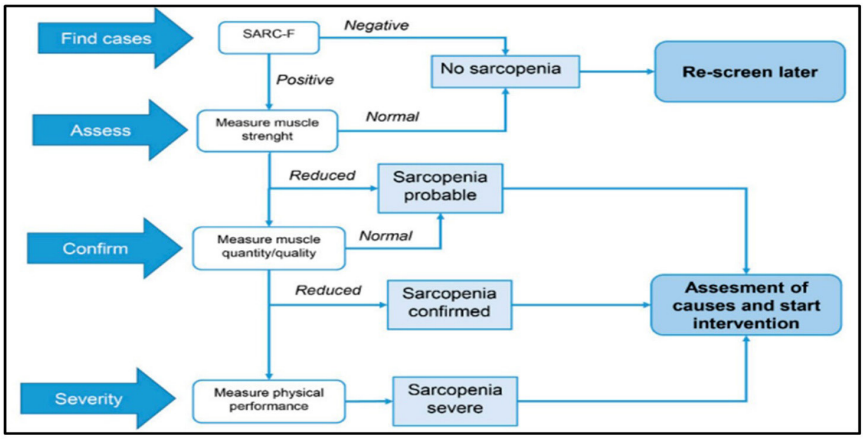

Figure 2. *EWGSOP-2 protocol for sarcopenia diagnosis. Discover the situation, determine the diagnosis, and determine the severity*

With the effect of the Covid -19 pandemic in its extraordinary states, rapid and effective diagnosis and treatment gained importance for primary health care services on digital health platforms. In digital health, -MAPP (Remote-Malnutrition APP-Remote Malnutrition Application) has been developed and its application has been made available. The protocols used clinical screening tools MUST and SARC-F to determine muscle wasting and nutritional risk. "MUST (Malnutrition Universal Screening Tool) and the abbreviation SARC-F are based on the initials of English words. These; Strength is power (Strength), Assistance with walking, Rise from a chair, Climb stairs, falling (Falls)" (Pekcan, 2022).

Sarcopenic Obesity

There are more studies demonstrating how nutrition can help treat and prevent sarcopenia. In some circumstances, sarcopenia and malnutrition need to be distinguished. Often, body mass is not a factor in the link between age-related muscle mass decrease and muscle strength. Sarcopenia obesity is defined as a rise in fat mass accompanied by a reduction in muscle mass. The definition put out is a shortage of skeletal muscle in comparison to fatty tissue (Prado et al., 2012). In addition to obesity, which is defined as an abnormal or excessive buildup of fat, elderly people are at a significant risk of developing sarcopenia, which is a progressive and

generalized loss of muscle mass and function (Batsis and Villareal, 2018; Eglseer et al., 2023). Based on previously discovered genome-wide significant SNPs associated with hand grip strength, appendicular lean mass and walking speed, 78 pleiotropic genomic predictors of sarcopenia were identified and characterized based on the UK biobank cohort study. Out of the 78 SNPs, two polymorphisms were linked to type 2 diabetes (T2D), and 55 polymorphisms were linked to body fat %, indicating that sarcopenia, obesity, and T2D share numerous risk alleles (Semenova et al., 2023). Compared to obesity or sarcopenia alone, sarcenic obesity has been more reliably identified as a sign of disability (Baumgartner et al., 2004). Although it might vary by gender and ethnicity, the frequency of sarcopenic obesity rises sharply with age and mostly depends on the condition's underlying criteria (Johnson et al., 2017; Batsis et al., 2017).

Age-related declines in skeletal muscle mass and function can coexist with fat gain, which promotes the initiation and progression of sarcopenic obesity. Individuals with obesity, regardless of age, may experience this. Obesity-related oxidative stress, inflammation, and insulin resistance may result in loss of muscle mass and function because of conditions like insulin resistance (Hong ve Choi, 2020; Donini et al., 2022).

There is loss of muscle mass in diets that produce low protein consumption when nutritional monitoring and follow-up are not carried out following bariatric surgery targeting adipose tissue in obesity (Sherf-Dagan et al., 2019; Pekar et al., 2020). Sarcopenic obesity (SO) can also be present after weight loss or in the uncontrolled cycling of body weight, especially in middle-aged and young people who are obese due to acute and chronic disorders. Detection method for sarcopenic obesity evaluation are given in Figure 3. The evaluation of people suspected of having SO will be divided into two stages: screening and diagnosis. The latter will enable the sickness to be staged on two different levels (Donini et al., 2022).

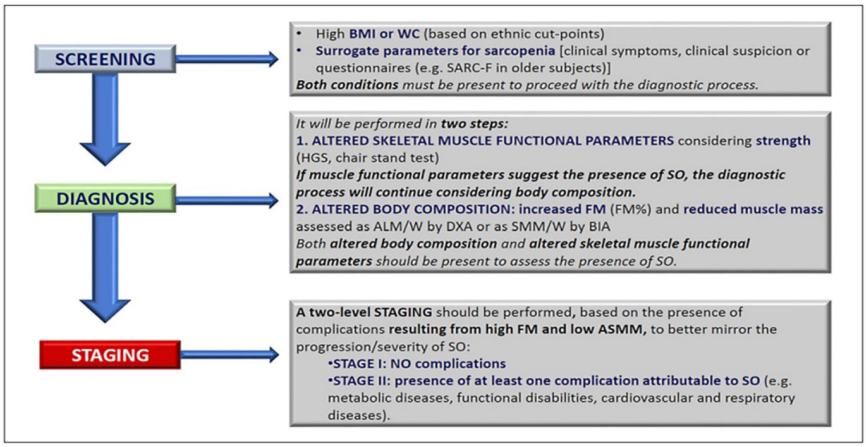

Figure 3. *Detection method for sarcopenic obesity evaluation*

It demonstrates that while physical activity recommendations should take precedence in cases of sarcopenic obesity, an appropriate calorie intake that satisfies the requirements is likely to be useful in preventing sarcopenia (Park et al., 2023). According to Batsis and Villareal (2018), calorie restriction has not yet been proven to be an effective dietary treatment for sarcopenic obesity. It takes careful medical monitoring and meal planning, frequently with the assistance of a certified dietitian with experience in this population, to maximize protein consumption while limiting calories (Okamura et al., 2022).

Sarcopenia can also develop from malnutrition, which can be caused by severely inadequate food consumption. Malnutrition causes accelerated aging, although a low protein and calorie diet can cause sarcopenia (Sieber, 2019; Murawiak et al., 2022). While it causes a decrease in muscle functionality in most of the elderly, it especially targets the elderly with a Body Mass Index (BMI) less than 18.5 kg/m^2 (Liquori et al., 2018; Toplar et al., 2022). According to a recent study, people who ate more than the recommended amounts of protein and calories had a lower risk of developing sarcopenia than people who did not get enough of those nutrients (Park et al., 2023).

A new systematic approach suggests that older persons with sarcopenia have a history of eating insufficient amounts of low-calorie foods that are lower in macronutrients and micronutrients than those consumed by older adults without sarcopenia. Additionally, research has demonstrated that sarcopenia weakens the muscles used for swallowing and is recognized as a risk factor for dysphagia (de Sire et al., 2022). Sarcopenic dysphagia is a swallowing condition brought on not only by sarcopenia of

the swallowing muscle but also of the entire body. Sarcopenia dysphagia was at risk due to aging, poor muscular mass, and decreased tongue pressure (Fujishima et al., 2019; Abe et al., 2023). When Brates et al. (2022) demonstrated a link between the risk of sarcopenia and malnutrition and fatigue related to swallowing and eating. All of these aggravate sarcopenia over time by lowering food intake. To enhance nutritional status, adequate nutrient intake is necessary (Santiago et al., 2021; Jesadaporn et al., 2023). Iatrogenic sarcopenia, which arises from nutritional deficiencies and inactivity, has been associated with appropriate rehabilitation measures that can be tailored to the severity of the condition (Kakehi, 2022). Although the length of each stage depends on the degree of the disease's invasion, inflammation, and severity, examples of their timing are provided. At every stage, proper diet and suitable exercise therapy should be used to prevent iatrogenic sarcopenia, which can become worse in cases of malnutrition (Table 3) (Kakehi et al., 2022).

Table 3. *Exercise and nutrition at various stages of care*

Stage	Nutritional therapy	Exercise therapy	
		Objective	Interventions
Hyperacute care[a]	Intake: less than 70% of the target energy goal Protein: 1.3 g/kg/day for protein	Minimizing the loss of physical function, muscle mass, and strength	Early movement, electrical stimulation of the muscles, or a bedside ergometer
Acute care[b]	Intake: not to exceed 20–25 kcal/kg/day	To keep physical function, muscle mass, and strength	increasing daily steps during gait training establishing a goal step count while using a pedometer
Subacute care[c]	Intake: 25–35 kcal/kg/day Protein: 1.2–1.5 g/kg/day	To improve physical function, muscle mass, and strength	Aerobic exercise and weight training Patients suffer motor function loss and muscle weakness, thus an activity regimen needs to be planned to prevent injuries[e].
Convalescent care[d]	Intake: more than the target energy to match the activity level	To improve physical function, muscle mass, and strength	Aerobic exercise and weight training

Appropriate Diet in Sarcopenia

Sarcopenia has no suitable clinical therapies (Wang et al., 2022). Currently, there is no medication that can be used to treat or prevent this illness (Bruyère et al., 2022). The relationship between nutrition and muscle mass, strength, and function in older persons is supported by mounting research, despite the fact that diet is a changeable and modifiable factor influencing sarcopenia (Yokoyama et al., 2021). Sarcopenia-related gene bioinformatics analysis in the UK Sarcopenia risk alleles were discovered in the Biobank cohort to be connected to fatigue, declines in the past year, neuroticism, frequent alcohol consumption, smoking, television viewing time, greater salt, white bread, and processed meat intake. Additionally, protective alleles are associated with higher levels of bone mineral density, serum testosterone, IGF1, and 25-hydroxyvitamin D levels, height, intelligence, cognitive performance, educational attainment, income, physical activity, coffee consumption, and healthier eating (muesli, cereal, wholemeal or whole grain bread) (Semenova et al., 2023).

In the older population with sarcopenia, an exercise program along with the prescription of nutritional supplements enhanced patients' muscle mass, enhancing muscle strength and walking performance (Luo et al., 2017). Sarcopenia that was both confirmed and severe was linked to poor dietary diversification (Kiuchi et al., 2023). These results raise the possibility that by enhancing diet quality, sarcopenia can be postponed or even avoided.

Protein Intake

Muscles play an important role in our daily routine work. In humans, skeletal muscles contain approximately 40% of the total body weight in 50-75% of all body proteins (Frontera and Ochala, 2015). Muscle mass is difficult to maintain without appropriate interventions due to pathological, pathophysiological and pathoanatomical conditions (Voulgaridou et al., 2023). Protein energy balance is an important determinant of healthy aging (Boirie et al., 2014). A balance between muscle protein anabolism and catabolism (Abiri et al., 2017) provides the maintenance of muscle mass. According to Frontera and Ochala (2015), these processes are susceptible to elements like disease, hormonal balance, physical activity, injury, and nutritional condition. Age-related muscle response reduction impairs the regulation of turnover. Muscle protein synthesis declines by 30% with aging (Park et al., 2018). Sarcopenia patients lose more muscle mass as they age due to a decreased anabolic response to protein synthesis (Kouw et al., 2017; Santos et al., 2019). In order to preserve skeletal muscle mass in the elderly, it's critical that they consume enough protein in their diet. Loss of muscle mass alters the metabolic and protein turnover of skeletal mus-

cle tissue, which can trigger the onset and development of other disorders (Masanés et al., 2017; Cruz-Jentoft et al., 2020).

Dietary protein recommended for adults stimulates muscle protein synthesis by providing the needed amino acids. Protein intake for adults is 0.8 grams per kilogram per day. This protein intake falls short of stimulating protein synthesis for the elderly (Keller, 2019). Protein intake for European Society of Clinical Nutrition and Metabolism (ESPEN) in older adults should be at least 1g/kg/BW/day (Volkert et al., 2019). In order to stimulate muscle mass synthesis at the highest level and create a feeling of satiety in the elderly, the source of dietary protein and the amount of intake in meals are important (Tieland et al., 2018; Toplar et al., 2022). A higher protein intake is required to stimulate protein synthesis in older individuals compared to younger adults (Bauer, 2011). Protein intake of 1.1 g/kg per day is recommended if there is no kidney failure or any contraindication to restricting protein intake (Dreyer and Volpi, 2005). Persistent renal failure When the risk of sarcopenia in the early stage of chronic renal failure is more serious than the risk of developing end-stage renal disease, intake of more than 1.3 g/kg per kg should be avoided, but adequate energy intake should be provided with physical activity in addition to protein intake (Barreto Silva et al., 2022). The dosage for healthy individuals should depend on their nutritional state, level of physical activity, and the outlook for their present disease, according to ESPEN (Volkert et al., 2019). Both the quantity of protein and how it is distributed throughout the day matter. Less protein is consumed in the morning and at lunch than in the evening meal, according to research. Increased total protein consumption throughout the day was connected to higher protein intake at breakfast and lunch without reducing daily total protein intake. Between the main meals, protein intake should be spread equally (Verreijen et al., 2021; Voulgaridou et al., 2023). Interventions for dialysis patients that combine carbs, protein, and fat seem to be more effective than those that only use protein, showing that increases in muscle composition can take up to 48 weeks (Barreto Silva et al., 2022). Nutrition-related variables against sarcopenia include the quality of the protein consumed as well as the usage of dietary supplements like HMB and creatine monohydrate (Cannataro et al., 2021). The association between consumption of animal and plant proteins and muscle mass index was examined in a study on older women to determine the quality of their protein intake. A stepwise regression study revealed that the only other independent predictor of muscle mass index was the consumption of animal protein ($R^2 = 0.19$; $p = 0.008$). Vegetable protein intake does not preserve muscle mass index as well as animal protein does (Lord et al., 2007). Naturally, it is advised to give children three main meals in a balanced manner in order to encourage this protein synthesis at the high-

est level (Bauer, 2011). This protein intake offers the crucial amino acids needed for the synthesis of new muscle protein. Leucine, one of the necessary amino acids, has been found to be correlated with skeletal muscle index values, increased grip strength, and physical performance (Voulgaridou et al., 2023; Rondanelli et al., 2021).

Leucine

The senior population with sarcopenia will have an improvement in quality of life by enhancing physical performance, muscular mass, and strength when oral nutritional supplements with a high leucine content and high quality protein are combined with resistance exercise, according to studies (Kim et al., 2012; Shahar et al., 2013; Luo et al., 2017; Volkert et al., 2019; Liao et al., 2019; Santiago et al., 2021; Jesadaporn et al., 2023). While the majority of studies showed that increasing protein synthesis while maintaining lean mass increased lean mass, the study in which leucine-enriched whey was given in conjunction with resistance training intervention showed no improvement in sarcopenia and fragility, and no gain was seen in muscle strength analysis (Rondanelli et al., 2020). However, in a recent systematic review, resistance exercise at least five times per week in combination with 3 grams of essential amino acids or 22-36 grams of whey protein supplementation daily also resulted in gains in skeletal muscle mass and total lean mass in sarcopenic older adults, as well as strength and stability gains, and synergistic effects on other quality of life indicators have been observed (Hernández-Lepe et al., 2023). Leucine, one of the branched-chain amino acids, is more potent than isoleucine and valine for promoting muscle protein synthesis (Martins et al., 2017; Mısıroğlu ve Köse, 2023). Leucine has significant regulatory effects on protein turnover and speed in skeletal muscles by increasing protein synthesis (De Bandt, 2016; Martnez-Arnau et al., 2019). This is because leucine rapamycin complex 1 (mTORC1) is directly used as a signaling molecule in muscle through activation at its mammalian target. Leucine supplementation given to elderly people with sarcopenia improved muscular protein synthesis, according to a randomized controlled clinical trial (Martnez-Arnau et al., 2020). Whey protein, which contains 13 grams of leucine per 100 grams, has been used as a leucine-containing supplement in the majority of randomized placebo-controlled studies (Hamarsland et al., 2017). The drink containing 4 grams of protein and 2.15 grams of leucine produced more myofibrillar protein synthesis than the drink containing 4 grams of 2.1 grams of leucine twice a day for six days (Yamashita et al., 2022). 34 grams of protein and 6 grams of leucine were observed to be sufficient to increase muscle protein synthesis in senior people in their study on undernourished elderly adults (Jesadaporn et al., 2023). This study found that giving malnourished older persons a high-protein, leucine-rich oral supple-

ment over the course of a 12-week period boosted physical performance without causing any negative side effects. Malnutrition can be eliminated, which leads to gains in muscle growth and strength as well as an improvement in bodily functions. Leucine has clear benefits when taken orally in conjunction with a high-protein diet, but additional research is required to establish the ideal dose to take in addition to resistance exercise. According to the international recommendations, eating 30 grams of leucine and 3-25 grams of protein with each of our three main meals promotes muscle growth by halting the elderly's loss of lean mass (Bauer et al., 2013; Rondanelli et al., 2020).

BCCA Support in Sarcopenia

The utilization of branched-chain amino acids for the treatment of sarcopenia is debatable. The cirrhosis of the liver causes portal hypertension. Due to increased catabolism brought on by cirrhosis and this portal hypertension, calorie intake decreases, which leads to malnutrition and sarcopenia (Merli et al., 2019; Gazda et al., 2023). As the organ's reaction to the body's requirements declines, sarcopenia gets worse in cirrhotic patients (Kitajima et al., 2018; Cengiz, 2019). Branch-chain amino acids (BCCA) are assumed to be advantageous because they are involved in the pathophysiological processes that preserve lean mass in some illnesses, such as liver cirrhosis. Patients with liver cirrhosis who participated in a randomized controlled trial received BCCA every day for six months. In sarcopenic patients with compensated and early decompensated cirrhosis, it has been demonstrated that the addition of BCCA to exercise did not increase muscle mass, functional indices of sarcopenia, or quality of life when compared to the placebo group. There is no evidence that BCCA by itself can treat sarcopenia. Branch-chain amino acid therapy is influenced by dietary and background protein intake (Le Couteur et al., 2020).

Omega 3 and antioxidants

A major contributing factor to frailty-related sarcopenia, an age-related NCD, is likely to be inflammation. Due to their direct impact on muscle catabolic and anabolic signaling pathways and connection to muscle wasting, CRP, IL-6, and TNF- are inflammatory cytokines linked to sarcopenia (Bian et al., 2017; Wang et al., 2017). Chronic inflammation may worsen muscle wasting (Pan et al., 2021). The dose-response connection was used to explain how the Dietary Inflammatory Index (DII) and Sarcopenia were related in the meta-analysis of the link between the two. According to the findings, sarcopenia risk is increased by pro-inflammatory diets whereas it is decreased by anti-inflammatory diets. They can prevent sarcopenia by increasing their intake of these components of anti-inflammatory diets (Diao et al., 2023). According to clinical investigations, patients with sar-

copenia have significantly higher CRP values than those without sarcopenia (Fujikawa et al., 2017). Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), two long-chain omega-3 polyunsaturated fatty acids (omega-3 LCPUFAs), reduce inflammation and make it more soluble, preventing the loss of muscle mass and strength linked to aging, sarcopenia, and fragility (Vatic et al., 2020; Troesch et al., 2020).

According to a research of sarcopenic aged women, the risk of sarcopenia decreases when omega-3 PUFA levels rise; however, there is inadequate data to link omega-6 PUFA levels to sarcopenia risk (Cacciatore et al., 2023). Omega-3 LCPUFA supplementation is promising in the treatment of sarcopenia because more research is demonstrating that these fats have an anabolic effect on the metabolism of skeletal muscle (Robinson et al., 2018; Dupont et al., 2019). Xu et al. (2022), they shared that n-3 PUFA supplementation for 6 months could beneficially affect physical performance, lipid profiles and muscle strength in elderly people, and that this could be one of the strategies to be used in the prevention of sarcopenia. Its impact on preserving lean body mass and lowering the risk of sarcopenia in the elderly is unknown due to a lack of data. There is a need for more randomized controlled studies of various lengths and dosages. Although there is no established medical cure for this ailment, sarcopenia sufferers may benefit from an anti-inflammatory diet (Papadopoulou, 2020; Papadopoulou et al., 2023). In contrast to saturated fatty acids, which can exacerbate inflammation, dietary n-3 fatty acids have anti-inflammatory properties (Calder et al., 2017; Antonopoulou et al., 2021). However, up until now, the majority of studies examining the impact of nutrition in sarcopenia have concentrated on single meals. Foods are combined when eaten. Individual foods or foods may not have as much of an impact as a diet plan since foods interact with one another and have an impact on health (Papadopoulou et al., 2022; Detopoulou et al., 2022). Given the findings, the compatibility of this diet with the Mediterranean diet has given rise to the hypothesis that, because of its anti-aging benefits in older people, it may have beneficial effects on sarcopenia (Mazza et al., 2021; Papadopoulou et al., 2023). Higher triglyceride levels have been linked to sarcopenic obesity and are proof of this, according to cross-sectional studies (Kim et al., 2023). Okamura et al. (2022) have researched the impact of hydrolyzed goiter gum on sarcopenic obesity. There were two study groups, one fed a diet high in fiber and the other low in fiber. The group took a soluble fiber called goiter gum, and this boosted their grip power and muscle mass. It raised the amount of short-chain fatty acids present in serums and feces. In terms of immunity, it decreased the proportion of inflammatory cells while increasing the fraction of anti-inflammatory cells in the small intestine. The researchers reported that goiter gum has a preventative impact on

sarcopenic obesity even though their experiments were the first to examine the effect of goiter gum.

Mediterranean diet

The Mediterranean diet's impact on sarcopenia has been researched since it contains beneficial fats. The Mediterranean diet is a dietary strategy that discourages use of dairy and meat products while encouraging consumption of monounsaturated fatty acids from olive oil, n-3 fatty acids from fish and plant-derived foods, vegetables, fruits, nuts, and whole grains (Abbatecola et al., 2018; Melekoğlu et al., 2020; Parlak, 2023). In adherents to the Mediterranean diet, the Mediterranean diet has been said to be associated with less loss of physical function in aging and frailty (Ntanasi et al., 2018; Capurso et al., 2019). Lower hand grip strength and a higher risk of sarcopenia were linked in one study to lower Mediterranean diet adherence ratings (Cacciatore et al., 2023). The Mediterranean diet and diet had a good impact on muscle hypertrophy and function, but the effects on muscle strength were less obvious. There is no proof to support the idea that the Mediterranean diet prevents sarcopenia (Papadopoulou et al., 2023). Future research is required to ascertain whether dietary changes, such as the Mediterranean diet, improve muscle strength in older people. There is a need for additional research given the constraints.

Vitamin D

Calcium homeostasis and bone metabolism are both influenced by vitamin D. In the liver, vitamin D is converted to 25-dihydroxyvitamin D, which is then converted to 1,25-dihydroxyvitamin D in the kidney (Lee et al., 2014). The active form of D vitamin, 1,25-dihydroxycholecalciferol, also known as calcitriol, is crucial for the intestinal absorption of calcium, magnesium, and phosphate. The process of absorbing calcium is crucial for us. By preserving calcium kinetics, dietary calcium intake promotes muscular strength (Fatima et al., 2019; Kirk et al., 2020).

According to Munns et al. (2016), vitamin D insufficiency is recognized as a global health issue. Measurement of serum concentrations of circulating 25 (OH) D is utilized in the assay in clinical practice for the evaluation of vitamin D (Holick et al., 2011). Hip fractures, overall mortality, and the onset of sarcopenia have all been linked to low vitamin D levels (Caristia et al., 2019; Papadopoulou et al., 2022). The risk of developing a number of diseases is now known to be positively correlated with vitamin D shortage and insufficiency. Remelli et al. (2019) evaluated the scientific and clinical evidence in favor of the link between vitamin D and an elevated risk of sarcopenia in older persons. One of the lowered biomarkers for sarcopenia is vitamin D (Papadopoulou et al., 2022). Vitamin D has an impact on the vitamin D receptor (VDR). By increasing muscle growth,

vitamin D promotes muscle strength and athletic performance by binding to the VDR receptor on muscle fibers (Shuler et al., 2012; Remelli et al., 2019). Studies are lacking because it is difficult to detect VDR protein, but it is known that vitamin D participates in the calcium phosphate pathway in the skeletal muscle system and that vitamin D supplementation aids in increasing VDR concentration as an age-related relationship (Ceglia et al., 2015; Şengün, 2019). It has been demonstrated that vitamin D encourages the initial rise in the cross-sectional area of skeletal muscle fibers and inhibits myostatin expression, which is a significant contributor to the degeneration of the healthy structure of muscle tissues and the inability of those tissues to carry out their normal functions (Remelli et al., 2019).

Myocytes manufacture and release the myokine known as myostatin, which stops muscle growth by blocking the PI3K/AKT/mTOR pathway (Soto et al., 2023). The number and width of type II muscle cells, particularly type IIA, are influenced by vitamin D. Acceleration, deceleration, sprinting, and jumping are examples of short-duration, high-intensity exercise behaviors that specifically require type IIA cells to create rapid muscle contraction rates (Agergaard et al., 2015; Papadopoulou et al., 2022).

According to a study looking at the impact of vitamin D on older adults with hand osteoarthritis, this potent steroid may assist to some extent lessen sarcopenia and the disability caused by hand osteoarthritis (Marks, 2023). The prevalence of sarcopenia was higher in men with hip fractures, and vitamin D deficiency was discovered to be more common in hip fracture patients in the studies looking at hypovitaminosis D, which compared vitamin D deficiency and sarcopenia in elderly patients who underwent hip fracture surgery and in patients who underwent elective primary total hip arthroplasty (Kim et al., 2021). The incidence of osteoporotic fractures in the older population can be decreased by vitamin D treatment (Chapuy et al., 2002; Bee et al., 2013).

Administration of vitamin D can boost muscular growth and strength, and it may be especially helpful in preventing and treating sarcopenia. Vitamin D supplementation did not always lead to an improvement in the muscle function evaluated, according to a meta-analysis of 16 randomized, controlled trials on postmenopausal women that looked at the effects of vitamin D supplementation on muscle performance. The indices of general muscle strength, grip strength and muscle strength, were not increased by vitamin D treatment (Tabrizi et al., 2019; Uchitomi et al., 2020). Gkekas et al. (2021) systematically reviewed the best available evidence on the impact of oral vitamin D alone or with protein supplementation on muscle strength and mass performance in patients with sarcopenia. Their findings demonstrate that vitamin D plus protein supplementation increases muscle strength in sarcopenia patients. There was no discernible impact on performance. In

a different trial, vitamin D administration paired with exercise and protein dietary supplements significantly improved grip strength in sarcopenia patients. Despite evidence that vitamin D administration may increase muscle mass and function, this finding was not recognized since it lacked statistical significance (Cheng et al., 2021). In order to verify the impact of vitamin D supplementation on the markers of muscle function of isolate and protein supplements, additional research is required to ascertain the ideal amount and period of administration. In the meta-analysis study by Chang and Choo (2023), the use of whey protein, leucine, and vitamin D supplements effectively and significantly improves appendicular muscle mass in patients with sarcopenia, independent of any physical activity program, without considering whether a physical exercise program combined with nutritional supplementation. It has been found that it can increase. However, neither the grip strength nor the physical performance were much improved.

Physical Exercise and Resistance Training with Sarcopenia

Whether or not obesity is a factor, exercise plays a fundamental role in avoiding the onset of sarcopenia. Exercise, particularly resistance and cardiovascular training, has been shown to be helpful in clinical investigations (Gan et al., 2018). Regular physical activity is essential for good aging and helps older persons avoid issues including pain, diminished mobility, and fragility (Eckstrom et al., 2020). The World Health Organization (WHO) has taken steps to promote senior people's awareness of proper nutrition and exercise as well as to increase their physical activity levels. This is due to the elderly population's rapid growth. among order to assure active aging among the elderly, which is a focus of WHO, universities were founded on several campuses in Turkey in 2002. It intended to promote physical exercise in these colleges to avoid future issues. Physiological issues will be avoided as a result, and disease recurrences will be reduced (Altun et al., 2022). According to the European Working Group on Sarcopenia in Elderly 2, treatment should focus on consuming enough protein and getting enough exercise. It has been suggested that the ideal ratio of protein consumption to exercise should be combined with an active lifestyle (Cruz-Jentoft et al., 2019). According to Dent et al. (2018), resistance-based exercise should be prescribed for sarcopenia. Sarcopenia risk was inversely related for women but not for men in a study that examined the relationship between healthy eating and sarcopenia without intervention by looking just at the food frequency consumption record in the elderly (Ghoreishy et al., 2023). In order to improve sarcopenia, a combination of dietary therapy and a thorough exercise regimen that incorporates resistance training is more beneficial than either intervention alone (Chen et al., 2020). Combining a higher protein diet with exercise can accelerate the decrease of body fat (Eglseer et al., 2023).

Resistance Exercise in Sarcopenia

In order to combat the negative effects of sarcopenia in older persons, resistance exercise (RE) is advised as the main form of therapy (Dent et al., 2018; Hurst et al., 2022). Resistance training is a successful treatment for sarcopenic obesity in people over the age of retirement (Eglseer et al., 2023). Exercises that require the muscles to work against a weight are referred to as resistance exercises. Weight, strength, or resistance training are other names for it (Chodzko-Zajko et al., 2009). Resistance training enhances muscle strength and mass in middle-aged and older persons, according to randomized controlled studies for the use of the therapy to treat sarcopenia (Grgic et al., 2020). When the condition before and after training was evaluated, it was shown that resistance exercise generated significant changes in the expression of 26 genes (out of 73 relevant genes) in human skeletal muscle. This highlights some of the advantages of strength training for sarcopenia prevention and treatment (Zhao et al., 2022; Semenova et al., 2023).

The optimal prescription for resistance exercise

There is virtually little data supporting the best prescription for resistance training. In the absence of an appropriate exercise dose and routine, it is challenging to realize the potential benefit of resistance exercise. Only when the dose is suitable for older persons can exercise have a therapeutic impact (Hurst et al., 2022). Resistance training adaptation is unique to a person's physiological stress. Because of this, each person should have their own unique exercise dose prescribed. Depending on the level of intensity and frequency of exercise, all these characteristics are combined to establish the dose of exercise. This has been shown to have been disregarded in earlier investigations.

It is confirmed that many programs, including successful studies in clinical research, are shorter, less intense, and erroneously administered without relying on evidence (Royal College of Physicians, 2016; Witham et al., 2020). This is true even for the programs in which this exercise training is utilized regularly. In the UK, exercise programs for older adults with sarcopenia and frailty are deficient in frequency, duration, and disease progression, and workouts for the sarcopenia patient group lack individualization, according to a 2019 UK-wide survey by Offord et al (2019). Retrospective investigations reveal the findings of cross-sectional research that sarcopenic individuals had a higher risk of falls and fractures compared to non-sarcopenic adults, according to the systematic review and meta-analysis by Yeung et al (2019). According to Yeung et al. sarcopenia is associated favorably with fractures and falls. A key risk factor for falls in older persons is decreased standing balance (Muir et al., 2010). Exercise

programs may combine various exercise programs due to the increased risk of falls and decreased cardiopulmonary fitness, which have a significant impact on how sarcopenia and physical handicap in the elderly are related (Chien et al., 2010). Resistance training has been shown to be effective in the treatment of sarcopenia in studies, but it is not yet routinely used in clinical settings. Compared to other workout programs, resistance training has shown to be more expensive. A new type of consensus is the absence of standards for standardized methods and the underrecognition of pharmacological therapy in the prescription of exercise (Sayer and Cruz-Jentoft, 2022).

CONCLUSION

Despite extensive ongoing research on pharmacological treatments for sarcopenia, there are no licensed medications or acceptable clinical procedures to treat the disease. Supplementation with whey protein, leucine, and vitamin D can considerably improve appendicular muscle mass in sarcopenic patients who are weak in muscle strength and function. When combined with a physical training routine, this can increase muscular strength and function. There have been inadequate studies to determine appropriate intakes, doses, and durations of therapy for resistance exercise. When paired with nutritional therapy, programs combining resistance training show promising outcomes, but the effect sizes are inadequate. To deliver the benefits indicated, future research must expand the number of studies that base their efforts on both resistance training programs and dietary advice.

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CHAPTER 3

EFFICIENCY AND COST EFFICIENCY OF HOSPITALS IN TURKEY[1]

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Introduction

One of the consequences of the zeal of societies to live at a high level of welfare is competition. The essence of competition is the issue of who will have access to resources scarcely, more, or abundantly. Hence, competition is directly associated with resource consumption. Resource consumption also increases in environments where competition heats up. Besides, unequable and rapid consumption poses life-threatening risks. In today's world where limited resources are being depleted rapidly, the major and most vital problem of humanity is to be able to access sufficient resources in the future. In order to overcome this problem, considering the effects of competition, costs must be minimized and resources should be planned and allocated properly.

Nowadays, as in every institution, the crucially of resource planning and how to distribute resources most accurately increases day by day in hospitals that use a significant part of the resources allocated to the health sector. Studies that deal with various issues such as determining the quality of the service delivered in hospitals, how to overcome the challenges encountered in the production process, how to allocate resources the most accurately, and in which areas to make improvements are generally based on the efficiency indicator. Efficiency is directly associated with resource utilization. In its simplest sense, efficiency is performing a task correctly In this regard, efficiency is to achieve the maximum output that can occur with an available resource or to realize the output level with the minimum use of resources. Due to the functioning of hospitals, their output is not always easily predictable. Thus, as a result of a number of arrangements to be made in the inputs, information related to the efficiency of the resources can be obtained.

Efficiency is closely associated with costs, as it is related to the optimal use of available resources. Given the issue of reducing costs to a minimum, information that will enable to reach maximum output with minimum resources is entailed. The capacity to produce by keeping the costs at a minimum determines the cost-efficiency. By comparing the efficiency scores with the costs, information is obtained about how accurately and at what cost the resource planning is performed, and in which domains the resources should be funneled primarily for the most accurate planning. The cost of the service provided in hospitals established by incurring huge costs is also high. This situation forces hospitals not only to be efficient but also to be cost-efficient. Today, where resources are quite limited, the issue of minimizing costs is of great importance for all hospitals, regardless of their ownership. How to make the right resource allocation with minimum cost is determined in the light of the information obtained as a result of the efficiency and cost-efficiency analyses conducted together.

The aim of this study is to determine the efficiency and cost-efficiency of hospitals in 81 provinces of Turkey by grouping them on the basis of provinces. There is a wide variety of studies in the literature that seeks to determine the efficiency or cost-efficiency of hospitals. Studies are aimed at determining the efficiency of a particular clinic (Gynecology clinic, emergency department, intensive care unit, etc.), one or more regional hospitals or hospitals specified based on their ownership. Hence, in these studies, the comparison of hospitals in terms of resource utilization or cost distribution was limited only to the hospitals that were the subject of the study. On the other hand, it is crucial to make generalizations, especially when comparing hospitals on a country basis or comparing certain groups of hospitals both within themselves and with different hospital groups. The overall assessments made across the country to determine the efficiency of the hospitals reveals the most optimal resource utilization for the relevant hospitals, while the generalization of cost-efficiency studies reveals the urgency priority of resource allocations by considering all hospitals in the country. Therefore, this study differs from other studies in the literature in that it involves all hospitals across the country. Although the studies conducted with certain hospital groups provide information for the relevant hospital groups to some degree, they do not reflect the general framework for the country in general. For this reason, hospitals cannot compare themselves with all other hospitals throughout the country, and the priority level of urgencies cannot be determined accurately in terms of resource allocation. In the current study, all hospitals in the country are assessed in terms of their efficiency and cost-efficiency to eliminate this deficiency identified in the literature and to offer an overall assessment for hospitals. Yet, hospitals were categorized by the provinces and analyzed, due to the data constraints faced.

When the literature is reviewed, it is noticed that Data Envelopment Analysis (DEA) is typically used to determine efficiency in the health sector (Lavers and Whynes, 1978; Borden, 1988; Chang, 1998; Kirigia et al., 2002; Temür and Bakırcı, 2008; AlShayea,2011; Androutsou et al., 2011). ; Du, Wang et al., 2011; Ichoku et al., 2011; Atmaca et al., 2012; Bayraktutan and Pehlivanoğlu, 2012; Gülsevin and Türkan, 2012; AlRefaie et al., 2013; Bal and Bilge, 2013; Jones, 2013; KawaguchiTone and Tsutsui, 2013; Fiallos, 2014; Köse, Uçkun, and Girginer, 2014; Çelik and Esmeray, 2014; Cheng et al., 2015; İswanto, 2015; Şenel and Gümüştekin, 2015; Girginer and Çalışkan, 2016; Kutlar and Salamov, 2016 ; Şenol, 2017; Almiman, 2018; Berk and Çerçioğlu, 2018; Kıraç et al., 2018; Şenol et al.,2019; Yılmaz and Şenel, 2019; Kılıçarslan and Güçlü, 2019; Habib, 2020;Yüksel, 2020; Boğa and Kayahan , 2021; Pereira et al., 2021; Chiu et al., 2022). In these studies, variables such as the number of physicians, the

number of nurses, the number of allied healthcare personnel, the expenditures on medications and medical supplies, the number of pharmacists, the number of patients, the number of beds occupied per day (on average), the total salary paid, the non-salary expenditures, the number of discharged patients, the number of inpatients, the number of general outpatient clinics, the number of special examinations, the number of laboratory tests and the number of x-ray imaging services were used. When the studies are examined in general terms, it is noticed that studies on cost-efficiency (Linna, 1998; Linna et al., 2006, Mathiyazhgan, 2006; Durnek, 2010; Furukawa, 2010; Medin, 2011; Mleşnite & Bocşan, 2016; Soares, 2017; Özdin et al., 2019; Torun et al., 2020; Çayırtepe and Kavak 2020) are conducted more commonly abroad than domestically.

The current study was designed to analyze the efficiency and cost-efficiency of all hospitals (public and private) in 81 provinces in Turkey by the provinces. In the study, hospitals can compare their success in obtaining output according to their inputs compared to other hospitals across the country, and if there are improvements that need to be made, they can find many hospitals that they can emulate and which are the most suitable for their own conditions in terms of resource utilization patterns, owing to their organizational similarities. As the hospitals that are the subject of the study are grouped on the basis of provinces, the results obtained encompass the whole country. Interpretations made for any province concern all hospitals in that province.

Design of the Study

The study was conducted to assess the efficiency and cost-efficiency of hospitals in 81 provinces of Turkey by grouping them on the basis of provinces. The main and sub-objectives of the research design determined for this purpose are listed as follows.

Main Objective: To rank the hospitals in 81 provinces of Turkey according to their efficiency through the DEA

Sub-Objective: To offer improvement suggestions for inefficient hospitals, based on the results of the DEA.

Sub-Objective: To rank the provinces where hospitals are located, based on their efficiency through gray relational analysis (GRA).

Sub-Objective: To rank the important variables in the efficiency

Sub-Objective: To make a cost-efficiency analysis and to rank the provinces with cost-efficient hospitals

Sub-Objective: To rank the important variables in cost-efficiency

Efficiency Analysis

The DEA, which is frequently utilized in the literature, was used for efficiency analysis. DEA is a linear programming-based method suitable for measuring the relative efficiency of decision-making units that convert similar inputs into similar outputs. As a result of the analysis conducted-through this method, an efficiency score ranging between 0 and 100 is assigned to each decision-making unit. The decision-making unit, which uses the input-output combination most optimally, has an efficiency score of 100 and is included in the reference set by determining the efficiency limit. Decision-making units with efficiency scores ranging from 0 to 100 are considered inefficient relative to the decision-making units in the determined reference set. Relatively inefficient decision-making units can reach the information that they can be efficient after how much improvement they have made, by choosing the most appropriate decision-making unit for them in terms of resource usage among the efficient reference set.

The decision-making units used in the study are hospitals. However, since the hospitals are grouped and analyzed on a provincial basis, all considerations made cover the hospitals in the relevant province as a whole. It is not always easy to plan the outputs of the hospitals due to their organizations and operations. On the other hand, hospitals can easily enhance their efficiency as a result of decreased-oriented improvements in their inputs. Hence, in the study, the input-oriented, output maximization-targeted BCC model was used when determining the efficiency rates.

The input and output variables used were determined as a result of detailing the literature study. Input variables used in the literature are generally divided into sub-domains such as labor force (specialist physician, general practitioner, nurse, other health personnel, etc.), machinery and equipment(medical device, number of rooms, number of beds, etc.), capital (health expenditures, investments, etc.), medicine and medical equipment, technology (machines, recording systems, etc.) and physical facilities (hospital establishment location, etc.). The input variables used in the study include the total number of physicians, the number of nurses, the number of allied healthcare personnel, and the ratio of medication sales value. Output variables used in the literature vary depending on the services provided in hospitals (number of visits to the physician, number of outpatient examinations, number of surgeries, etc.). The output variables used in the study include the number of surgeries performed and the number of visits to the physician.

The input and output variables used in the study were determined based on the data in the 2016 Health Statistics Yearbook published by the Ministry of Health. All the variables in the DEA model established in the light of the

determined variables are proportioned to the total population obtained from the 2016 Turkish population information published by the Turkish Statistical Institute and the 2016 Turkey Migration Report published by the Turkish Republic Ministry of Interior General Directorate of Migration Management. Thus, the impact of the population on the variables was included in the study. All the data were used by multiplying by 10.000 to prevent the small numbers encountered as a result of the ratios to make the study difficult. The established DEA model is presented in Table 1.

Table 1: Input and Output Variables of the DEA Model

	Input Variables	Output Variables
DEA Model	Total number of physicians per 10,000 people	Number of surgeries performed in 10,000 people
	Number of nurses per 10,000 people	Number of visits to the physician per person
	Number of allied health personnel per 10,000 people	-
	The ratio of medication sales value	-

Calculations in the formula of (Related variable / total population) * 10.000 were used to reflect the effects of the population on the data of the input and output variables used in the study. The input variable of medication sales value ratio was obtained by dividing the sum of the 2016 data by the total number of applications to the physician of the relevant province in the Medication Sales Value by Years and Selected ATC-1 Groups table presented in the 2016 Health Statistics Yearbook (Total medication sales value / Total number of applications to the physician in the relevant city).

Results of Efficiency Analysis (DEA)

The efficiency map colored based on the results of the efficiency analysis conducted throughout Turkey is shown in Figure 1. Dark blue color indicates the provinces with inefficient hospitals (0 - 79% efficiency score), and light blue color indicates the provinces with hospitals far from being efficient (80% - 89% efficiency score), while yellow color indicates the provinces with hospitals close to being efficient (90% - 99.9% efficiency scores) and the green color indicates the provinces with efficient (100% efficiency score) hospitals.

Figure 1: Efficiency Map of Turkey by Provinces

According to the DEA result, the number of provinces with efficient and inefficient hospitals in Turkey was determined. Table 2 shows how many times the resource utilization patterns of the provinces with efficiency were referenced by the inefficient hospitals, which variables should be the most necessary for the improvements required for the inefficient hospitals and the geographical distribution of the efficient hospitals.

Table 2: Results of Efficiency Analysis

DEA Model	Number of Efficient/ Inefficient Provinces	The Most Referenced Provinces	Performance improvements	Geographical Distribution of Efficient Provinces	Metropolitan Distribution of Efficient Provinces
	24 provincial hospitals are efficient and 57 provincial hospitals are inefficient	Uşak, Şanliurfa, Rize	The medication sales value ratio is decreasing, and the number of surgeries is increasing	Marmara Region and Southeastern Anatolia Region	14 metropolitan hospitals are efficient

According to the results of the DEA model, in which the number of nurses, the number of alliedhealthcare personnel, the total number of physicians, and the ratio of medication sales were used as input variables, and the number of surgeries and the number of applications to physicians

per person as output variables, hospitals in 24 provinces (Bursa, Mardin, Şırnak, Ankara, İstanbul, Gaziantep, Şanlıurfa, Kilis, Rize, İzmir, Bartın, Niğde, Samsun, Denizli, Amasya, Trabzon, Edirne, Uşak, Tekirdağ, Balıkesir, Erzurum, Isparta, Adana, and Yalova) have been found to be successful in terms of resource utilization, while hospitals in 57 provinces are found to be unsuccessful. The hospitals of the provinces that are most referenced by these inefficient hospitals are located in Uşak, Şanlıurfa, and Rize. Kilis and Erzurum hospitals, meanwhile, were not taken as reference by inefficient hospitals, although they were efficient. The improvements that were considered necessary in the utilization of the resources were determined as decreasing the medication sales value variable and increasing the number of surgeries variable. The provinces with efficient hospitals are generally situated in Marmara (Bursa, Istanbul, Edirne, Tekirdağ, Balıkesir, Yalova) and Southeast Anatolia (Mardin, Şırnak, Gaziantep, Şanlıurfa, Kilis, Adana) Regions. Of the provinces with efficient hospitals, 14 were metropolitan.

Suggested Improvements Based on Efficiency Analysis Results

Some of the hospitals that were found to be inefficient as a result of the efficiency scores obtained by DEA, in which hospitals located in 81 provinces of Turkey were grouped according to provinces, and the improvement rates required for these hospitals to be efficient are presented in Table 3. The provinces in this table, where the hospitals are located, have been chosen randomly to set an example, considering that they are closest to the efficiency limit (100%), less close, or farthest away.

According to Table 3, hospitals in Kocaeli and Sakarya provinces have efficiency rates of 99.3% and 98.5%. The hospitals of these provinces are considered relatively successful in using their resources as they were very close to the 100% efficiency score.

The total number of physicians per 10,000 people (16), the number of other health personnel per 10,000 people (16), and the ratio of medication sales value (981) in Kocaeli hospitals are sufficient to reach the efficiency limit. As a result of an improvement towards a decrease of 6.21% in the number of nurses per 10,000 people (19), the number of applications to the physician per person (9) increased by 0.75% and the number of surgeries performed per 10,000 people (611) increased by 3.67%, hospitals of this province will become more efficient.

Table 3: Improvements that Need to be Made for Some Provinces with Inefficient Hospitals in the DEA Model

Provinces	Efficiency Score (%)	Potential Improvement Rates					
		Input Variables				Output Variables	
		Total number of physicians per 10,000 people (%)	Number of nurses per 10,000 people	Number of allied health personnel per 10,000 people	The ratio of medication sales value	Number of applications to a physician per person (%)	Number of surgeries performed in 10,000 people (%)
Kocaeli	99.30	0	-6.21	0	0	0.75	3.67
Sakarya	98.50	0	0	-1.58	0	1.54	13.53
Eskişehir	92.40	-0.37	-1.57	0	0	8.25	8.25
Ağrı	84.10	0	0	-10.51	-54,47	18,89	33,86
Van	75,10	0	0	0	-16,16	33,20	33,20
Hakkari	57,50	0	-4,28	-19,06	-84,15	73,85	80,34

Hospitals in Hakkari province have an efficiency score of 57.5%. The lowest efficiency score obtained as a result of DEA belonged to the hospitals of this province. Hence, these hospitals are in the lowest rank in Turkey in terms of their success in using resources efficiently. Although the total number of physicians per 10,000 people in this province (9) is sufficient, for efficiency, improvements should be made in the number of nurses per 10,000 people (12) by -4,28%, in the number of allied health personnel per 10,000 people (14) by -19,06% and the rate of medication sales (14476) by -84.15%. As a result of these improvements, an increase of 80.34% and 73.85%, respectively, is expected in the number of surgeries performed among 10,000 people (172) and the number of visits to the physician per person (4).

Cost Efficiency Analysis

In addition to performing a task efficiently, it is the subject of cost-efficiency analysis to be able to perform it with minimum cost. Cost efficiency analysis shows the degree to which the activities are carried out correctly with minimal cost. For this reason, this analysis is carried out in order to make the proper resource planning simultaneously with the correct budget planning. By looking at the results of the analysis, it can be seen which of the inefficient hospitals can become efficient with less cost. Besides, this order of priority determines the order of resource distribution.

The cost-efficiency analysis consists of three stages. In the first stage, it is necessary to be able to express the ratio of the cost of hospitals on a provincial basis within the total cost (Current health expenditure + investment health expenditure). Hospital costs were obtained by deriving from shared data since hospital cost data were not available on a provincial basis. In the second stage, the ratio of the relevant hospitals to the total costs

of the other hospitals that are the subject of the analysis is calculated, while in the last stage, the cost-efficiency ratio is reached by proportioning the efficiency scores of the relevant hospitals with the ratio obtained.

It has been assumed that theoretically, the more hospitals a province has, the more health investment expenditures are made. In addition to this, it is thought that the hospitals belonging to the relevant province receive a share of this expenditure in proportion to the total number of hospitals throughout the country since the investment health expenditure value represents the total investment health expenditure value in 2016 (7.216 million Turkish Liras). The costs of hospitals were calculated based on this information and assumptions.

The obtained cost-efficiency ratio value is directly proportional to the difficulty of inefficient hospitals to become efficient. Hospitals with a small cost-efficiency ratio can become more efficient with less transfer of funds. Hence, it is suggested that hospitals with a low cost-efficiency ratio should rank higher in resource allocation.

Results of Cost-Efficiency Analysis

Table 4, which has been prepared based on the cost-inefficiency and efficiency or cost-efficiency and inefficiency status of these hospitals, as well as the information on whether the hospitals that are determined to be cost-efficient or cost-inefficient according to the results of the cost-efficiency analysis are located in metropolitan cities, is presented below. The provinces in the table represent the general information obtained throughout the country. In addition to this, calculations for certain provinces are presented in the table to set an example. With the cost-efficiency (CE) ranking, the ranking obtained as a result of the analysis of the provinces where the efficient and some inefficient hospitals, which are the subject of cost-efficiency analysis, belong is indicated.

Table 4: Results of Cost-Efficiency Analysis

Cost Efficiency Analysis	Metropolitan Hospitals Are Often Cost-Inefficient	Non-Metropolitan Hospitals Cost Efficient	Hospitals That Are Efficient but Cost-Inefficient	Hospitals That Are Inefficient but Cost-Efficient
	Istanbul, Ankara, Izmir, Antalya etc.	Bayburt, Kilis, Bartin, Iğdır etc.	Istanbul, Ankara, Izmir, Bursa, Samsun etc.	Bayburt, Iğdır, etc.
Example of Efficient but Cost-Inefficient Hospitals: Bursa Province Hospitals	Bursa Hospitals Efficiency Rate: 100	Bursa Hospitals Cost: (7.216)*(39/1.510)=186.37	Bursa Hospitals Cost Ratio: 186.37/119.756=0.0015	Bursa Hospitals Cost-Efficiency Ratio: $1,5*10^{-5}$ 25. C.E. Ranking
Example of Inefficient but Cost-Efficient Hospitals: Bayburt Hospitals	Bayburt Hospitals Efficiency Rate: 70.48	Bayburt Hospitals Cost: (7.216)*(1/1.510)=4.77	Bayburt Hospitals Cost Ratio: $3.9*10^{-5}$	Bayburt Hospitals Cost-Efficiency Ratio: $5.6*10^{-7}$ 1. C.E. Ranking

According to the results of the cost-efficiency analysis, hospitals located in metropolitan provinces such as Istanbul, Ankara, Izmir, and Antalya are generally cost-inefficient. On the other hand, hospitals located in non-metropolitan provinces such as Bayburt, Kilis, Bartın, and Iğdır are generally cost-efficient. The hospitals of the provinces that are generally in the metropolitan category, such as Istanbul, Ankara, Izmir, and Bursa, are cost-inefficient, though they are efficient. Although these hospitals plan their resources properly, they cannot control their budgets or the budget allocated to them is insufficient to ensure efficiency. Therefore, accurate resource planning is achieved by incurring huge costs. In hospitals located in smaller provinces such as Bayburt and Iğdır, meanwhile, despite the inefficiency of the hospitals, cost efficiency is seen. This indicates that efficiency does not guarantee cost-efficiency or vice versa.

Bayburt hospitals are in 1st place in the CE ranking. It can be suggested that these hospitals can become efficient with fewer resources transfer compared to the inefficient hospitals in other provinces. Hence, based on the results of the analysis, the first hospitals to be reconsidered are the hospitals in Bayburt.

Ranking of Provinces by Gray Relation Analysis (GRA) in terms of their Efficiency and Cost Efficiency

GRA is used for decision-making and forecasting. The system that is the subject of the analysis includes variables (grey elements) about which there is a lack of information. GRA is used to determine the relationship between two gray elements or two subsystems of the analyzed system. The analysis reveals the similarities or differences of this relationship. This relationship, also called the gray relationship, expresses the change between gray elements or two subsystems and the type of relationship that develops accordingly. If the change occurs continuously and together, the relationship between the elements is high; however, if it does not occur continuously but together, the relationship between the elements is low (Uçkun and Girginer, 2011). In this analysis, a reference series (a province with efficient hospitals) is generated in line with the objectives pursued in the studied system, and other series (other provinces with efficient hospitals) are graded based on their similarity with this reference series. This value, which is determined as a result of an array of processes, is called the Gray relational degree.

In this section of the study, efficient hospitals are determined in the first section and the variables are listed among themselves according to their significance in determining this efficiency, through GRA. Similar rankings were made between the cost-efficient hospitals determined in the second section as well as between the variables, according to their significance in determining the cost-efficiency. The rankings were formed by grading the gray relation degrees (GRD) obtained through GRA from highest to lowest.

Table 5, which includes the first five and last five provinces in the ranking of the provinces with efficient hospitals based on the GRA, is as follows.

Table 5: Ranking of Efficient Hospitals

Provinces	GRD of the provinces with efficient hospitals	Ranking of provinces with efficient hospitals
Bartın	0,733341	1
Uşak	0,731754	2
Rize	0,729128	3
Yalova	0,727385	4
Amasya	0,703109	5
Isparta	0,574156	20
İzmir	0,531937	21
Erzurum	0,495197	22
İstanbul	0,490498	23
Ankara	0,459858	24

According to Table 5, the first five provinces in the ranking of the provinces with efficient hospitals are Bartın, Uşak, Rize, Yalova, and Amasya. These provinces were found to be efficient with an efficiency score of 100% as a result of DEA. Similarly, as a result of cost-efficiency analysis, hospitals belonging to these provinces were found to be cost-efficient. The fact that the abovementioned hospitals can balance their costs while using their resources properly confirms that their efficiency levels are ranked in the first place. On the other hand, it is noticed that hospitals in metropolitan provinces such as Erzurum, Istanbul, and Ankara, which are not cost-efficient, are in the last place in the efficiency ranking. Although the hospitals belonging to these provinces use their resources properly, they are ranked lower than both efficient and cost-efficient hospitals in the efficiency ranking due to the problems in cost policies. Because these provinces can achieve their efficiencies by incurring huge costs.

Table 6 shows the ranking of the variables that make up the DEA model according to their significance in determining efficiency. Rankings were determined by considering the values of the GRD from highest to lowest.

Table 6: Ranking of Variables According to Their Significance in Determining the Efficiency

Variables	Model		
		GRD	Ranking
Input Variables	The ratio of Medication Sales Value	0.955955	1
	Total Number of Physicians	0,842042	2
	Number of Nurses	0,678565	3
	Number of Allied Health Personnel	0,581694	4
Output Variables	Number of visits to the physician per person	0,531877	5
	Number of Surgeries	0.403094	6

According to GRA, the most significant variable in determining the efficiency of hospitals is the medication sales value ratio. This variable should be subject to a significant improvement, based on the DEA result. Medications and medical supplies, which are among the most critical inputs of hospitals, are rapidly depleted and must be used meticulously. Unnecessary, inefficient, or improper medication consumption should be avoided. As a result of the downward improvements to be made in this variable, many hospitals will become efficient. Hence, this variable is of great importance for efficiency. Other variables that are significant in determining the efficiency are the labor force resources such as the total number of physicians, the number of nurses, and the number of allied health personnel, respectively. These inputs are important in determining the efficiency according to their order in the table since the excessive or under-required number of labor force resources yields a negative result in terms of efficiency. In determining the efficiency, the number of visits to the physician per person and the number of operations performed variables were found to be less important compared to the other variables.

Table 7 below shows the first three provinces and the last three provinces, which were ranked among themselves through GRA, of the hospitals that were found to be cost-efficient according to the cost-efficient analysis. However, in order to make some comparisons, the rankings of the relevant provinces as a result of the cost-efficiency analysis have also been added to the table.

Table 7: Ranking of Cost-Efficient Hospitals

Provinces	GRD of the provinces with Cost-efficient hospitals	Ranking of provinces with cost-efficient hospitals (GRA)	Cost Efficiency Analysis Ranking
Bartın	0,710289	1	3
Uşak	0,707032	2	7
Yalova	0,702112	3	5
Iğdır	0,63331	10	-
Kilis	0,633049	11	2
Bayburt	0,553908	12	1

According to GRA results, the hospitals of Bartın Province rank first place in terms of cost-efficiency. Hospitals of Uşak and Yalova provinces rank in the second and third places. It is known that these hospitals, which are in the first three places, are also efficient with an efficiency score of 100%, according to the DEA results. Although hospitals in Bayburt and Kilis are ranked 1st and 2nd in terms of cost efficiency according to cost-efficiency analysis results, their cost efficiency is in the last place according to GRA (ranked 12th and 11th in GRA). This can be explained

by the inefficiency of hospitals in Kilis and Bayburt, according to DEA. Although hospitals in Kilis and Bayburt work with balanced costs, they are in the last place in the ranking of GRA, since they cannot plan their resources correctly. Hence, when it comes to ranking the cost-efficiency of hospitals, it is crucial whether they use the resources that are the subject of the efficiency correctly, as well as the size of the cost they use to ensure their efficiency.

Table 8 shows the ranking of the variables according to their significance in determining cost efficiency with GRA. Although the primary variables of cost-efficiency analysis are cost value, cost ratio, and efficiency score, since the variables that determine efficiency are the variables of the DEA model, the significance of these variables in determining cost efficiency can be stated.

Table 8: Ranking of Variables According to Their Significance in Determining the Cost Efficiency

	Model		
		GRD	Ranking
Variables	Number of Surgeries	0,737095	1
	Number of visits to the physician per person	0,715717	2
	The ratio of Medication Sales Value	0,644167	3
	Total Number of Physicians	0,504858	4
	Number of Nurses	0,434469	5
	Number of Allied Health Personnel	0,348726	6

According to the GRA results, the most important variable in determining the cost-efficiency of hospitals is the number of surgeries performed. The second and third important variables are the number of visits to the physician per person and the ratio of medication sales value. The most important variables in determining cost-efficiency, the number of surgeries, and the medication sales value ratio are the variables that require significant improvement as a result of DEA. Thus, efficiency rates increase as a result of the correct planning of these resources. Since rising efficiency rates have a positive impact on cost efficiency, it can be suggested that the variables that require more improvements in determining cost efficiency are more important.

Conclusion and Suggestions

The concept of health, which constitutes the basis of life from the past to the future, has equal significance for people of all languages, religions,

and races. Besides, being healthy and being able to access healthcare services in the fastest way without any problems is a consistent need for all humanity. Hospitals, where health care is provided, are complex organizations that are quite costly to establish and run. In hospitals where huge investments are made and resources are used up intensively, the continuity of this service is ensured by using resources properly and balancing costs, and distributing them correctly. For this reason, the most important objective of all hospitals, regardless of their ownership, is to perform the service provided at the lowest cost at the most optimum resource utilization level. Currently, hospitals can achieve the desired level of service by considering the results of efficiency and cost-efficiency analyses.

In the current study, hospitals in 81 provinces of Turkey were categorized based on the provinces, and their resource utilization and costs were analyzed through efficiency (Data Envelopment Analysis), cost-efficiency, and gray relational analysis.

In the first section of the study, efficient and inefficient hospitals were determined by grouping them on the basis of provinces, using data envelopment analysis. The number of times that efficient hospitals are referenced by inefficient hospitals in terms of resource utilization patterns and the required improvement rates for inefficient hospitals have been determined. In the second section, some hospitals that were selected as an example of efficient and inefficient hospitals were subjected to cost-efficiency analysis. As a result of the cost-efficiency analysis, cost-efficiency and cost-inefficient hospitals were determined and the necessity of channeling the resources to which hospitals with priority was mentioned. In the third section of the study, efficient and cost-efficient hospitals were listed among themselves through gray relational analysis. Ultimately, in order to reveal the significance of the variables used in the data envelopment analysis model in determining the efficiency and cost efficiency, the variables were listed using gray relational analysis.

According to the results of the efficiency analysis conducted with the data envelopment analysis, the hospitals in 24 provinces were efficient, whereas the hospitals in 57 provinces were not.The hospitals that are most referenced in terms of resource use by inefficient hospitals are located in the provinces of Uşak, Şanlıurfa, and Rize, respectively. Of metropolitan cities, the hospitals of 14 provinces were found to be efficient. Thus, almost half of the metropolitan cities across the country were found to be efficient. According to their geographical distribution, efficient hospitals mostly belong to Marmara Region and Southeast Anatolia Region. This result is not compatible with the result of the study conducted by Temür and Bakırcı in 2008. In the aforementioned study, it was concluded that the Marmara Region is a region with mostly inefficient hospitals. Variables that require

intensive improvements are the decrease in the sales value of medications and the increase in the number of surgeries, similar to the results of the study conducted by Bal and Bilge in 2013. Excessive or improper use of medications prevents access to this resource by people who really need to access it. For this reason, improper use of this resource is one of the leading causes of inefficiency across the country. In the study, it was concluded that the number of physicians, nurses, and allied health personnel, which were used as input variables, should be reduced and improvements should be made. This result is consistent with the results obtained in the studies by Kirigia, Emrouznejad and Sambo (2002), Mathiyazhgan (2006), Temur and Bakırcı (2008), Atmaca et al. (2012), Bal and Bilge (2013), Köse et al. (2014), Girginer and Çalışkan (2016) and Kılıçarslan and Güçlü (2019).

Based on the results of the cost-efficiency analysis, hospitals located in metropolitan provinces such as Istanbul, Ankara, Izmir, and Antalya are not cost-efficient. Hence, it is useful for hospitals in these provinces to reconsider their budget and cost policies. Although many metropolitan hospitals are efficient, they cannot be cost-efficient since they achieve this efficiency by incurring huge costs. Cutting-edge technologies and intensive labor resources used in metropolitan hospitals cause enormous costs. Inconsistent budget plans resulting from the inactivity of these cutting-edge technologies and current expenditures caused by the labor force create heavy financial burdens on hospitals. In addition to all these, similar to the results of Bayraktutan and Pehlivanoğlu's (2012) study, metropolitan hospitals are generally university and training and research hospitals for training specialist personnel, as well as for providing healthcare services. Therefore, as suggested by Linna (1998), if cost-efficiency is measured directly on the healthcare service provided, training and research hospitals show low performance. Because medical and nursing students are also factors of production. By definition, students are less productive; Graduate medical students' salaries are almost as high as professionals, despite using more time, materials, and tools for the same task as professionals. Hence, budget plans are made primarily for the purpose of training specialist personnel in universities and training and research hospitals. Besides, hospitals in provinces such as Bayburt, Kilis, and Bartın, which are generally not metropolitan cities, were found to be cost-efficient. Hospitals in Kilis and Bartın were also efficient. However, hospitals in Bayburt were found to be inefficient. The budget allocated to these hospitals is insufficient to achieve efficiency. Considering the cost-efficiency analysis, resources should be allocated primarily to the hospitals of Bayburt province. Thus, the relevant hospitals can become efficient at the same time. The presence of hospitals that are efficient and not cost-efficient or vice versa indicates that these two analyses cannot guarantee each other.

According to the results of the ranking of efficient hospitals among themselves by gray relational analysis, the hospitals located in the provinces of Bartın, Uşak, and Rize are at the top of the list. These hospitals are both efficient and cost-efficient. The efficiency of the provinces with efficient but cost-efficient hospitals, such as Istanbul, is at the bottom of the list.

The most important variables in the ranking of importance of variables in determining efficiency are the ratio of medication sales value, the total number of physicians, and the number of nurses, respectively. Generally, as a result of data envelopment analysis, it has been found that the variables that need to be made important improvements are at the top of the list.

According to the results of the ranking of cost-efficient hospitals among themselves by gray relational analysis, the first three hospitals are located in the provinces of Bartın, Uşak, and Yalova. Moreover,hospitals in these provinces are also efficient. It was concluded that the ranking of cost-efficient hospitals among themselves is closely associated with the efficiency score.

In the ranking made according to the significance of the variables in determining cost efficiency, the first three of them are the number of surgeries, the number of visits to the physician per person, and the ratio of medication sales value, in which remarkable improvements are suggested in the data envelopment analysis. The importance rankings determined by the gray relation analysis have resulted in reducing costs

All analyzes conducted within the scope of this study, covering all hospitals throughout Turkey, are based on a unique model in which input and output variables are determined by us within the framework of certain rules. Thus, it is possible to obtain varying results with models in which input and output variables are diverse in different studies. In similar studies to be conducted in the future, it is expected that the analyzes will generate more comprehensive results by diversifying the variables to be used as a result of a more transparent sharing of hospital data.

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CHAPTER 4

CURRENT CLINICAL APPROACH TO INTERSTITIAL NEPHRITIS

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Interstitium is the extraglomerular-extravascular space of the kidney, filling the area between the tubules. Diseases that involve the interstitum include acute interstitial nephritis, chronic interstitial nephritis, endemic nephropathies, plasma cell diseases and reflux nephropathy.

This chapter will mainly discuss interstitial nephritis with a focus to acute interstitial nephritis, as well as specific drugs and diseases that may cause intersitital nephritis.

Acute Interstitial Nephritis

Acute Interstitial Nephritis is generally a reversible entity which is characterized with hypersensitivity reactions. When related to drugs, the reaction is not dose dependent and may recur with repeat exposures to the causative agent. Drug induced AIN usually starts within 3 weeks following the start of the offending drug. In some situations, like sarcoidosis and tuberculosis, delayed hypersensitivity may result in granuloma formation. While both cellular and humoral immunity are involved in AIN pathogenesis, cellular immune response is more dominant (Raghavan R & Shawar S, 2017). Because of the effect of TGF-Beta, it may heal with fibrosis which makes the condition prone to chronic kidney disease.

Acute interstitial nephritis is found in 2-3% of all kidney biopsies (Praga & Gonzalez, 2010). Almost 15% of acute kidney injuries without a definitive etiology are AIN. 70-90% of all AIN are related to drug exposure. Two most common drug groups responsible for AIN are antibiotics (around 50%) and non-steroidal anti-inflammatory drug. Given that they are often used without a definite indication, proton pump inhibitors as a causative agent for AIN should also be noted. Among antibiotic related AIN, methiciline is the hallmark agent. In addition, beta lactam antibiotics, sulfonamides, fluoroquinolones and rifampin may also cause AIN. Other drugs that may cause AIN are furosemide, allopurinol, tyrosine kinase inhibitors and immune check-point inhibitors.

There are 4 proposed molecular mechanisms, for AIN pathogenesis (Sanchez-Alamo et al, 2023):

1- Acting as a hapten, the drug may bind to tubular basement membrane

2- With an antigen mimicry mechanism, the drug may be directed to interstitium and generate an immune response towards the antigen it resembles.

3- The drug may deposit itself to the interstitium and be kept there trapped.

4- By generating antibodies, the drug may form complexes with that

antibodies and be deposited in the interstitium.

As mentioned above, both cellular and humoral immunologic mechanisms may play role in the pathogenesis. In cellular pathway, Th1 cells are activated while in humoral pathway Th2 lymphocytes activates B lymphocytes to generate antibodies. Th2 dependent pathway also activates eosinophiles which play the dominant role in hypersensitivity reactions.

Pathology

Inflammatory infiltrates are generally seen in deep cortex and outer medulla. The infiltrate is mainly composed of T cells and monocytes. Plasma cells, eozinophiles and granulocytes may accompany these cells. T cells mainly affect distal tubules. Generally, there is no correlation between the degree of inflammation and etiology (Joss et al, 2007)

Occasionally, non-necrotizing granulomas may be encountered. When seen in the pathological preperations, these granulomas may be related to infections, sarcoidosis, sjogren disease or wegener disease. As a rule of thumb glomeruli are normal in AIN. However, in a very rare type of AIN that is presented with nephrotic syndrome, minimal lesion like pathology may be seen in electron microscopy.

Direct immuneflourescence is generally negative in AIN. However when the causative agent is non-steroidal anti inflammatory drugs or allopurinol, linear IgG deposition may be seen along the tubules.

Clinical manifestations and course:

Acute interstitial nephritis generally occurs a few days or weeks after the start of offending drug. It may occasionally occur after months as well. The patients may present with oliguria and flank pain. This is becuase of the stretching of the renal capsule as a result of parenchymla edema. Hematuria or pyuria frequently accompanies this symptom. While leukocyte casts may be seen, eryhtrocyte casts are almost never found. As a result of sudden loss in glomerular filtration rate, around one third of the patients need renal replacement therapy.

Among extra-renal manifestations are subfebrile fever, maculopapular rahs, arthralgia and eozinophilia (Clarkson MR et al, 2004). Hemaolysis with thrombocytopenia as well as high transaminase levels may also be seen. Serum IgE levels may be found elevated in some patients.

Duration and severity of acute kidney injury are the most important determinants of the reversibility of AIN. Creatinine does not decreasae to baseline levels in almost 40 to 50% of the patients.

Diagnosis

While eosinofilia is more sensitive as a hallmark of hypersensitivity reaction, eozinophiluria is a more specific finding for AIN. TNF-alpha and IL-9 levels may be found increased in urinalysis though these are not very specific to AIN. One of the most important clinical strategies to guide diagnosis is the resolution of AKI after witholding the offending agent. Apart from these direct or indirect indicators, gold standard to diagnose AIN is kidney biopsy. For patients whom a kidney biopsy may not be performed, Ga67 scintigraphy may be used to detect the renal parenchymal inflammation (Graham F et all, 2016).

In patients who are using multiple drugs, detection of the causative agent may be via lymphcoyte stimulation test and anti-drug antibodies. Anti-drug antibodies are generally used to diagnose rifampin related hypersensitivity.

Treatment:

Most important first step to treat AIN is withholding the offending agent. If renal functions do not tend to normalize in a week a course of steroid treatment should be commenced. Prendisolone or methy-prednisolone are usually given at 1 mg/kg/day dose. This dose of steroid should be continued for a maximum of 2 weeks after when the dose should be tapered. The total duration of steroid therapy should be planned for 4 to 6 weeks. When cases are resistant to steroids, mycophenolic acid may be tried in selected cases (Preddie DC et al, 2006).

For AIN that are related to infectious agents, this should be noted that pyelonephritis may co-exist because of concomitant parenchymla infiltration. Possible etiologies for infection related AIN are hantavirus, human immuno-deficiency virüs and leptospirosis. When detected, infections are treated with relevant antibiotics, antivirals or supportive therapy. Corticosteroids are generally not recommnded except fro tuberculosis cases.

Specific culprit agents:

Rifampin:

Rifampin related AIN generally occurs when patients are exposed to the agent repetitively. AIN is not expected in the first exposure. Gastrointestinal symptoms and myalgia may accompany renal findings. Hemolysis, thrombocytopenia and high transaminase levels may be seen (Manika K, et al ,2013). In some previous reported cases, antirifampicin antibodies were present. Kidney biopys reveals tubular injury and interstitial infiltration.

Allopurinol:

Allopurinol related AIN are generally seen in patients who already has chronic kidney disease. Skin rashes and high transaminase levels may also be present. In more severe cases Stevens-Johnson Syndrome (SJS) may also occur. Risks is higher in patients who posess HLA-B58. With hypersensitivity to allopurinal, systemic involvement with dermatologic findings may also be seen. The spectrum of symptoms and clinical findings change from Stevens-Johnosn Syndrome to Toxic Epidermal Necrolysis and DRESS (Drug reaction with eosinphilia and systemic symptoms) (Esposito AJ, et al, 2017). A comparison for differential diagnosis of skin hypersensitivity reactions can be found in the table below.

Disease	Stevens Johnson Syndrome (SJS)	SJS – TEN Overlap	Toxic Epidermal Necrolysis (TEN)	DRESS (Drug Reaction with Eosinophilia and Systemic Symptoms)
Time from drug use to symptoms	In 3 weeks	In 3 weeks	In 3 weeks	3 – 8 weeks
Dermatologic findings	Dusky eryhtema and pseudo-target lesions (<10% of total body) + mucosal lesions	Dusky eryhtema and pseudo-target lesions (11-29% of total body) + mucosal lesions	Bullae, epidermal detachment, (>30% of total body) Nikolsky sign is generally positive, mucosal involvement is severe	Generalized maculopapular rash, bullae, exfoliation
Systemic Involvement	Often (fever-malaise)	Often (fever-malaise)	Always (fever)	Fever, lymphadenopathy

Non-Steroidal Anti-inflammatory drugs (NSAID)

In AIN cases with NSAIDs, nephrotic syndrome may also be seen. Because of the anti-inflammatory characteristics of the drug itself, the disease self-limits itself and clinical manifestations are seen almost 6 months to 1 year later than start of the drug. NSAID related AIN is more frequent in females and in patients who are older than 50 yearsl old.

There is also another entity called as NSAID nephropathy. The spectrum of NSAID nephropathy involves glomerular diseases like minimal lesion disease and membranous nephropathy, tubulo-interstitial nephritis and papillary necrosis.

Proton Pump Inhibitors (PPI)

PPIs today are prescribed to large patient populations without a definitve diagnosis and justification. A link between PPI use and CKD has been previously suggested (Wu B et al, 2021) and PPIs may also result in interstitial nephritis. Symptoms in PPI related AIN are generally mild and nonspecific. AIN symptoms like malaise and fever are seldomly reported in less than 10% of PPI related AIN cases.

Immune check-point inhibitors

Inhibition of check-points in immune system activates T cells with the objective of canalizing these acivated T cells to kill diseased cells such as tumor cells. Chekcpoints that are inhibited by certain monclonal antibodies are programmed cell death-1 (PD-1), an inhibitory receptor; programmed cell death ligand-1 (PD-L1), a ligand of PD-1; and cytotoxic T-lymphocyte antigen 4 (CTLA-4), a competitive ligand for CD28. Examples to the monoclonal antibodies are: Nivolumab, Pembrolizumab (PD1), Atezolizumab (PD-L1) and Ipilimumab (CTLA-4). However, in some circumstances these activated T cells may affect normal tissues which results in adverse effects. These include colitis, rhabdomyolysis hepatitis, nephritis (Seethapathy H et al, 2021), pneumonitis and dermatological complications such as maculopapular rash, vitiligo, SJS.

In patients who are using immune check-point inhibitors, creatinine levels should be followed up for a period of least 6 months in intervals which will not exceed 2 weeks. This should be known that, whenever a dermatologic manifestation develops, risk of interstitial nephritis also increases. To treat interstitial nephritis, corticosteroids can be used in 1-2 mg/kg/day dosing. The dose should be tapered after a week to complete the therapy in 4-6 weeks. In corticosteroid unresponsive cases, anti-TNF agents (e.g. infliximab), micofenolic acid or calcineurin inhibitors (cyclosporine, tacrolimys) may be tried. As it has regulatory effect on Th17 cells, vitamin D treatment may be added to AIN treatment.

Granulomatous Interstitial Nephritis:

This type of interstitial nephritis has worse prognosis may have an oliguric course (Storrar J et al, 2019). Certain drugs, sarcoidosis, urate crystal nephropathy, Wegener granulomatosis and mycobacterial infections may cause granulomatous interstitial nephritis.

Diseases which may result in Interstitial Nephritis

Sarcoidosis:

Sarcodosis which is characterized by non-caseating granuloma formation also manifests with hypercalcemia and hypercalciuria (above 300 mg/dL in 24 hours urine collection). Hypercalcemia happens because of increased production of 1 alpha hydroxylase in the granulomas. Hypercalciuria may be responsible for nephrolithiasis and calcium precipitates may cause acute tubular necrosis. However, the most frequent renal pathology of sarcoidosis is interstitial nephritis with granuloma formation. Mild proteninuria (500-1000 mg/day) and sterile pyuria may be observed. As in other majority of AIN cases, treatment is with steroids (1mg/kg/day – maximum 60 mg). The tapering of steroid dose should be slower than usual and maintenance therapy should be extended to over 2 years. This is because, the relaps risk is increased in sarcoidosis related interstitial nephritis.

Sjogren's Disease

Interstitial nephritis in the course of Sjogren's disease may result in chronic tubular disfunction. When there's concomitant distal renal tubular acidosis, there may be severe hypokalemia. AIN related acute kidney injury generally well responds to steroids. If not, micofenolic acid or rituximab may be used as adjunctive treatment.

Systemic Lupus Erythematosus (SLE)

Though very rare, lupus may spare glomeruli and involves the tubules (Moyano Franco MJ et al 2009). Because of immunologic active characteristics of SLE, there will be immune deposits along the tubular basement membrane. Tubolo-interstitial nephritis of lupus may be treated with corticosteroids. Azathiopurine is another option. Because of the sparcity of the cases, experiences with other immuno-suppresive agents are limited.

IgG4-related disease

This disease is generally seen in male patients who are older than 50 years. Serum IgG4 levels are more than 144 mg/dL. Interstitial infiltration involves IgG4(+) plasma cells and there will be immune deposits along tubular basement membrane. In biopsy there may be a sharp border between affected and unaffected areas. Concomitant membranous nephropathy is occasionally observed. In imaging studies, there may be visible inflammatory masses. These masses may even cause uretheral obstruction. Interstitial nephritis of IgG4 related disease is generally responsive to corticosteroids. Cyclophosphamide and rituximab may be used in unresponsive cases or as steroid sparing agents.

Hypocomplementemic interstitial nephritis

This entity is characterized with low C3 and C4 levels. In addition to general tubulo-interstitial infiltration, there are IgG, C3 and C1q immune deposits in the interstitum and along the tubular basement membrane. This disease may be thought in the spectrum of IgG4 related diseases. However, repeat biopsies may reveal differen findings. Treatment is with steroids. For steroid resistant cases, rituximab may be preferred.

Malignancy related AIN

This is generally seen in the course leukemias and lymphomas. When treated with relevant chemotherapeutics and radiotherapy, they may resolve completely.

Idiopathic AIN:

The main representative of this pattern is TINU (Tubolo-interstitial nephritis and uveitis). Systemic symptoms such as fever and myalgia as well as optahlomologic findings like vision loss or uveitis may be observed. In kidney biopsies there is interstitial infiltration without any granuloma formations. Prognosis of this disease is excellent. Corticosteroids are the treatment of choice.

AIN in kidney transplant patients:

The main etiology of acute interstitial nephritis in kidney transplantation patients is acute rejection episodes. BK Virus infection may also cause AIN. Also, chronic use of drugs such as trimetoprim-sulfametoksazol may cause intersitital nephritis.

Chronic Interstitial Nephritis

Extended exposure to the offending drug or agent may result in progressive scarring of renal parenchyma. Main mediators are PDGF and TGF-Beta which are released from fibroblasts. Chronic interstitial nephritis is characterized with tubular atrophy, macrophage and lymphocyte infiltrations and interstitial fibrosis. Occasinally, this entity may be a result of glomerular diseases related proteinuria and as well as ischemia.

Major etiologies for chronic interstitial nephritis are:

- Drugs (e.g. Lithium, cyclosporine, tacrolimus, cisplatin)
- Metabolic (e.g.hyperuricemia, hypokalemia, hypercalcemia)
- Heavy metals (e.g. lead, cadmium, arsenic, mercury)
- Radiation

- Balkan Nephropathy
- Immune-mediated (SLE, Sjögren, sarcoidosis, Wegener, vasculitides)
- Vascular diseases (atherosclerosis)
- Transplantation (chronic transplant reaction)
- Hematologic disturbances (multiple myeloma, lymphoma, sickle cell disease)
- Progressive glomerular diseases (glomerulonephritis, diabetes, hypertension)
- Analgesic nephropathy
- Chronic obstruction
- Hereditary (nephronophytisis, polycystic kidney disease etc)
- Infections

They may be detected incidentally. Most common findings are hypertension and decreased glomerulara filtration rate. Proteinuria is less than 1 g/day. While leukocyte casts can be seen, eryhtrocyte casts are almost always absent. As eryhtropoetin production is also affected, anemia may be seen early in the disease course.

Tubular disfunction in chronic interstitial nephritis may manifes as proximal or distal renal tubular acidosis. Nephrogenic diabetes insipidus and salt wasting syndrome may be seen as a result of tubular dysfunction.

For treatment, exposure to possbile offending agents should be avoided as much as possible. If underlying disease is known (e.g. lupus, sarcoidosis etc), specific treatment for that disease must be planned. General treatments such as optimization of blood pressure with renin-angiotensin-aldosteron system inhibitors should be added.

Lithium Nephropathy

Lithium (Li) is an agent with narrow therapeutic index (0,6-1,25 mmol/L) and used for the treatment of bipolar disorder. When exceeds the therapuetic doses, Li may cause nephrogenic diabetes insipidus as well as acute and chronic Li nephrotoxicity. Biopsy may show interstitial fibrosis and tubular atrophy. There is not any specific treatment for Li toxicity. Dose should be decreased. Amiloride may decrease intracellular Li levels. Thiazide diuretics shouldn't be used as they may increase Li resorbtion and cause volüme contraction.

Anlagesic nephropathy

Analgesics may cause medullary ischemia. Main pathology of analgesic nephropathy is papillary necrosis. Secondary tubular atrophy, interstitial fibrosis and mononuclear infiltration may also be seen. Analgesic nephropathy is more frequent in female patient. Patients who had analgesic nephropaty have an increased risk for uro-epithelial transitional cell carcinoma.

Chronic uric acid nephropathy

This is also called as gouty nephropathy. When uric acid crystals accumulate in the medulla, they cause inflammation in their surrounding. Hypertension and vascular diseases may accompany uric acid nephropathy. Hyperuricemia may activate renin-angiotensin-aldosteron system and is also a risk factor for de novo chronic kidney disease. Allopurinol may be used to control uric acid levels; however, the starting dose should not exceed 50-100 mg/day. Please remember that allopurinol is itself an AIN causing agent. Dose may be increased if urate levels stays higher than target level.

Radiation Nephritis

Patients may develop radiation nephritis when exposed to 20-30 Gy of radiation for more than 5 weeks. First target of radiation is endothelium. Thrombotic microangiopathy may develop. First and the most common clinical finding is hypertension. Kidneys should be protected from radiation during radiotherapy.

Inflammatory Bowel Diseases

Especially in Chron's diseases calcium oxalate stones and amyloidosis may occur. However, there are reported interstitial nephritis cases that are related to amino-salicylic acid derivatives. Intersitital nephritis may resolve by withholding the drug, if not a course of steroid treatment may be prescribed.

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CHAPTER 5

TYPE 1 DIABETIC PANCREAS: A TANGLED STORY OF BETA-CELLS, EXTRACELLULAR MATRIX AND IMMUNE SYSTEM

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1. Introduction

Diabetes mellitus or simply diabetes is a chronic disease of the pancreas associated with elevated levels of blood glucose due to the body's inability to produce insulin or to effectively respond to it. Thus, plasma glucose and glycated hemoglobin levels are increased, and these indicators are used as diagnostic criteria for diabetes. Classification of this disease spans 4 major groups: Type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes and a special group of diabetes due to other causes. Former two groups are the most common ones, T2D having the highest prevalence (>90% of the cases). T2D is linked to insulin resistance in the responding cells and thus, high glucose levels. However, in T1D, insulin secreting beta-cells are actively destroyed by the immune system leading to the insulin deficiency which compromises glucose removal from the blood circulation resulting in the hyperglycemia (American Diabetes Association Professional Practice Committee, 2022). T1D has less incidence rate compared to T2D, yet it was previously reported that approximately 8.42 million people were living with T1D globally with 0.5 million new cases in 2021. Still, it is proposed that the overall number of patients for that year should be higher, as it is estimated that nearly 3.7 million missing cases including the ones due to mortality of the disease (Gregory et al., 2022). Driven by environmental and genetic factors, metabolic problems in the organs due to lack of insulin and harm caused by the glucose create complications and advance devastating outcomes of this disease (Saberzadeh-Ardestani et al., 2018).

In general consideration of the development of diseases, the function of extracellular environment must be understood to prevent or treat diseases. By increasing our understanding of this relationship, traditional treatment strategies can be revised, and new treatment modalities can be designed. As the body is not solely built with the cells, extracellular matrix (ECM) and body fluids are required for the integrity and sustainability of the multicellular systems. In the pancreas, cell-ECM interactions take part in the development, cell survival and management of insulin secretion (Kaido et al., 2004). The presence of ECM and its communication are not only limited to the endocrine cells and other tissue resident non-immune cells. This network is remodeled by immune system cells such as macrophages and can be a tract for leukocytes in which cell surface receptors such as integrins, leukocyte associated immunoglobulin like receptor 1, discoidin domain receptor 1 and 2, macrophage scavenger receptors, CD44, and secreted proteins including matrix metalloproteinases control this activity (Rowley et al., 2019). Additionally, in pancreatic tissue engineering, promising transplants can be achieved by mimicking this microenvironment with at least its dimensionality as well as designing immu-

noisolation barriers as the routes for immunoprotection and if possible, eliminate immunosuppressive drug use. For this reason, encouraging strategies including biomaterials and spheroid systems are focused on currently which are inspired from the native pancreas organization (Bal et al., 2019; Bal et al., 2022; Wang et al., 2021b). Thus, dynamic interaction between immune system, beta-cells and extracellular matrix plays crucial roles to maintain healthy state as well as push for the underlying mechanisms of pancreas related diseases. Following sections depict the role and crosstalk of each pillar of T1D to disseminate the complexity of the process.

2. Autoimmunity in T1D

The trigger for the onset of the T1D is numerous such as infective particles which activate IFN-α signaling in islets. Stressed beta-cells express IFN-α-STAT1-IRF7-MHC I complex axis to present self-antigens to $CD8^+$ T cells **(Figure 1)** (Jiang et al., 2022). Another MHC I feature involves hyperexpression of these cell surface proteins in correlation with other response markers such as protein kinase R and myxovirus resistance protein in T1D patients. The associated pathway of this alteration downregulates several proteins in the glucose transport, voltage-gated ion channels and vesicle transport (Apaolaza et al., 2021). Death of beta-cells also releases beta-cell specific antigens captured by conventional dendritic cells. These dendritic cells travel to the lymph node where they present peptides from beta-cell proteins such as chromogranin A, proinsulin, GAD65, IA-2 loaded onto MHC class II molecules to diabetogenic $CD4^+$ T cells (Amdare, Purcell & DiLorenzo, 2021; Lehuen et al., 2010). $CD4^+$ and $CD8^+$ T cells communicate with B cells to boost the immune activity and epitopes such as IA-2, insulin and GAD65 recognized by B cells fuel the immune system and secretion of the autoantibodies at the periphery (Herold et al., 2013; Smith, Simmons & Cambier, 2017). In mice, immunoglobulin Gs (IgGs) drive neutrophils to release CRAMP (LL-37 in humans) which activate plasmacytoid dendritic cells along with self-DNA and DNA-specific IgG by the induction of TLR9-MyD88 pathway resulting in IFN-α secretion (Diana et al., 2013). Moreover, $CD4^+$ cells release IFN-γ and IL-2 upon antigen presentation to stimulate $CD8^+$ T cell cytotoxic activity against beta-cells (Espinosa-Carrasco et al., 2018a).

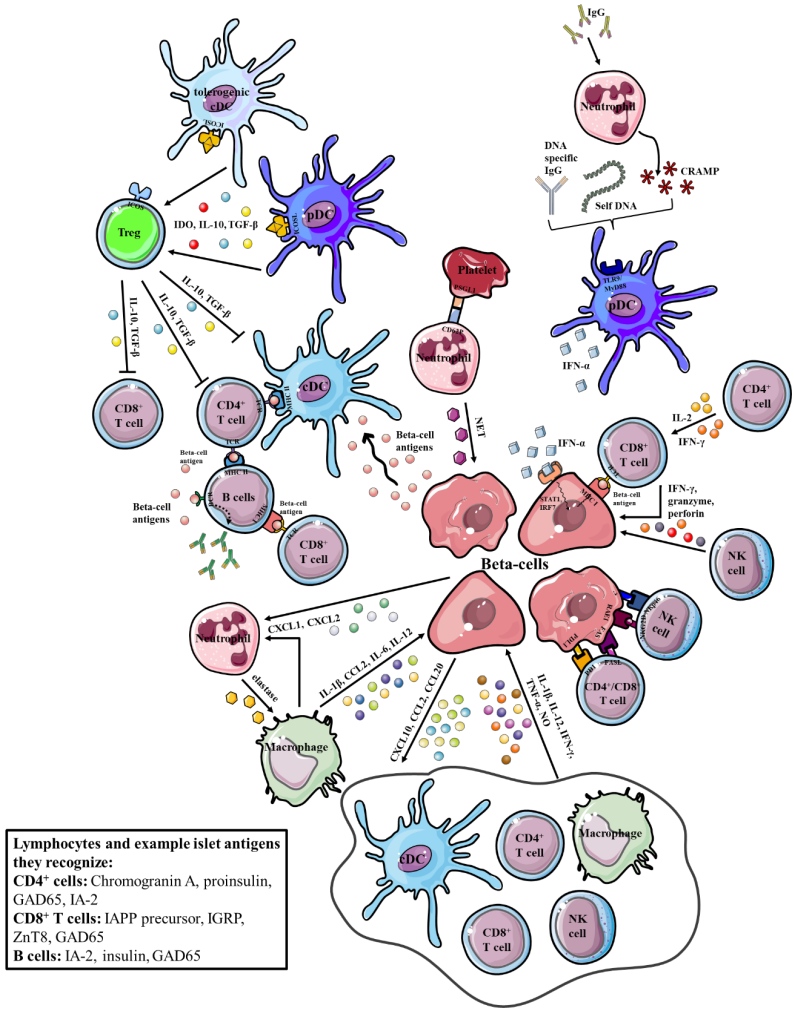

Figure 1. *Autoimmune network of T1D. Example soluble and cell-cell interactions are represented. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.*

To defend the body, macrophages, lymphocytes, and other immune system cells secrete IL-1β, IL-12, IFN-γ and TNF-α to alleviate immune reaction and induce apoptosis of the beta-cells. Meanwhile, stressed beta-cells produce large amounts of CXCL10, CCL2, CCL20 and other chemokines to attract the macrophages and others to the pancreas (Lehuen et al., 2010). The evidence regarding the effects of resident macrophages in T1D indicates that this subset has numerous effects in the progression of T1D, as lack of the resident macrophages lessens the recruitment $CD4^+$

T cells and dendritic cells as well as autoantigen presentation (Carrero et al., 2017). Diverse studies have shown human beta-cell epitopes such as preproinsulin signal peptide, IAPP precursor, IGRP, ZnT8 and GAD65 presented by MHC class I molecules on beta-cells are recognized by TCR of diabetogenic $CD8^+$ T cells (Belle, Coppieters & Herrath, 2011; Pugliese, 2017). For instance, Anderson et al. studied the frequency of preproinsulin reactive $CD8^+$ T cells and observed that it can reach above 40% and reported that various MHC class I molecules, especially HLA-A*02:01, used for the presentation of epitopes such as signal peptide derived from preproinsulin (Anderson et al., 2021). Similarly, leader sequence of IAPP precursor in recent onset T1D patients also belongs to the epitope class of HLA-A*02:01 amplifying the added risk of T1D (Panagiotopoulos et al., 2003). In the pancreas, beta-cells are actively killed by these diabetogenic T cells and natural killer (NK) cells by IFN-γ, granzymes and perforin, and TNF-α, IL-1β and NO of macrophages (Lehuen et al., 2010; Tomita, 2017). These processes are of utmost importance to T1D, and in fact, elastase derived from neutrophils catalyzes signature events of macrophage pro-inflammatory cytokine (e.g. TNF-α, IL-1β, MCP-1, IL-6, IL-12) delivery to induce apoptosis and autoantigen release from beta-cells. In turn, it is established that macrophage and beta-cell chemokines such as CXCL1 and CXCL2 are responsible for neutrophil infiltration to the pancreas (Shu et al., 2020). In the other branch of neutrophil attack, citrullinated histone binding to platelet TLR2/TLR4 or autoantigen-IgG immune complex binding to platelet FcγRIIA stimulates platelets. CD62P (P-selectin) of neutrophils and PSGL1 of activated platelets traffics these cells to the pancreas to release neutrophil extracellular traps injuring beta-cells (Petrelli et al., 2022).

For the time being, Treg derived IL-10 and TGF-β attempts to inhibit immune system cells to preserve beta-cells. In the lymph node, tolerogenic conventional dendritic cells and plasmacytoid dendritic cells positively manipulate Tregs with factors such as ICOSL, IDO, IL-10 and TGF-β for immune tolerance (Lehuen et al., 2010). Beta-cells express IDO-1 in the physiological conditions, though the level diminishes as T1D advances (Anquetil et al., 2018). In the body, Tregs generally control immunosuppression by acting on mainly T and B cells through inhibitory cytokines (e.g. IL-10, IL-35, TGF-β), cytolic activity (granzyme and perforin mediated), metabolic malfunctioning by IL-2 depletion, inhibition of dendritic cell maturation by MHCII and CD80/86 signaling manipulation, adenosine-dependent inhibition and PDL1-PD1 mediated apoptosis **(Figure 2)** (VignaliCollison & Workman, 2008). Thus, in beta-cell replacement therapy, activation or attraction of these cells to the area of graft is considered as a promising approach (Bal et al., 2019; Oran et al., 2019). However, Tregs

fail to detain the immune attack, as they are dysfunctional, most probably linked to the genetic factors that are classified as T1D susceptibility loci such as IL2RA, IL2, PTPN2, CTLA4, and IL10, but they are also associated with Treg function. Subsequently, IL-2 signaling impairment, reduced TCR repertoire and diversity, and continual exposure to inflammatory mediators hamper Treg-based immunosuppression (Bettini & Bettini, 2021).

Additionally, considerable level of other cell surface ligand-receptor interaction occurs between beta-cells and immune arm of the disease. For instance, FASL of $CD4^+$ or $CD8^+$ T cells can direct FAS expressing beta-cell fate to the apoptotic direction (Amrani et al., 2000; Thomas, Trapani & Kay, 2010). However, PDL1 of beta-cells negatively regulate diabetogenic T cell attack. Nevertheless, NKG2D and RAE1, and NKp46 interaction with an unknown partner communication leads to beta-cell damage and degranulation of NK cells. During this struggle, ICOSL of dendritic cells and ICOS of Tregs prompts immunosuppressive activity as one of the resorts against beta-cell death (Lehuen et al., 2010).

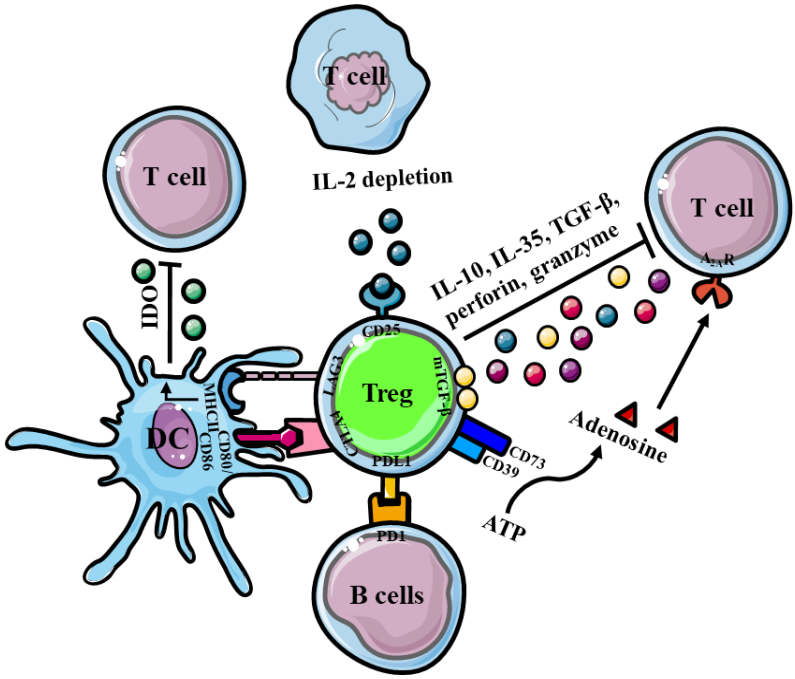

Figure 2. *Treg strategies shaping immune actions on T and B cells. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.*

Overall, this complex chain of events establishes hallmarks in the sig-

nificant loss of beta-cells and manifestations of adverse effects in the whole body.

3. Hyperglycemia and immune system

3.1. Macrophages

Hyperglycemia also cripples body defense, as it has harmful effects on immune cell function. Among the immune system cells, macrophage proliferation, (metabolic) activity and polarization are strongly influenced by hyperglycemia. A recent study proved that long-term exposure to high glucose does not interfere with bone marrow derived macrophage differentiation, viability and proliferation. Yet, in these macrophages, TNF-α and IL-1β productions were elevated, synthesis of IL-6 and IL-12p40, NO production (after LPS+IFN-γ stimulation), phagocytosis, glycolytic capacity and reserve were adversely affected. This points out the potential contribution of macrophage impaired function to the sensitivity of diabetic patients to infections and reduced wound healing (Pavlou et al., 2018). Similarly, TNF-α and IL-1β secretion were elevated in human monocytes when they were exposed to high glucose concentration (Moganti et al., 2017). In another study, hyperglycemia activated glucose transporter-1 expression and the resulting glycolytic pathway diminished the interaction between Rheb and GAPDH activating mTOR signaling. This process, in turn, led to senescence-associated secretory phenotype and inflammaging with periodontal tissue damage caused by macrophages in diabetic mice (Wang, et al., 2021a). Moreover, reprogramming of glucose metabolism and epigenetics in macrophages under hyperglycemic conditions contributes to the development of atherosclerosis (Edgar et al., 2021). In atherosclerosis lesions, synergistic activity of glucose-oxidized LDL and hyperglycemia stimulates macrophage proliferation (Lamharzi et al., 2004).

3.2. Dendritic cells

Dendritic cells are bridges between innate and adaptive immune systems as antigen presenting cells to provoke T cell response and conversely, to stimulate tolerance (Almeida & Everts, 2021). Nonetheless, loss of glycemic control in T1D disturbs dendritic cell function. A recent study showed that in tolerogenic dendritic cells, costimulatory molecule, CD86, increased and immunosuppressive function was diminished with high glucose (Thomas, et al., 2020). So far, *in vitro* studies strengthen the support of the impairing role of abnormally high glucose levels in the generation of less proinflammatory dendritic cells from monocytes while differentiated monocyte-derived dendritic cells and potentially $CD11c^{+}$ dendritic cells of adipose tissue are likely to be more immunogenic (Brombacher & Everts,

2020). In hypercholesterolemia, $CD11c^+$ dendritic cells can retain their antigen presentation capacity (Packard et al., 2008), yet in poor glycemic control, tolerogenic T cell function decreases (Dáňová et al., 2017). It was proposed that glucose toxicity guides dendritic cells to the cornea and interaction of these cells with subbasal nerve plexus causes nerve damage (Leppin et al., 2014). In another attempt to discover the effects of glucose toxicity on dendritic cell phenotype, it was observed that scavenger receptors such as SR-A, CD36 and LOX-1 are upregulated with the consequence of enhanced capacity to uptake oxidized LDL. This potentially enhanced lipid and antigen presentation as well as conversion into foam cells being beginning step of the atherosclerosis. With the exposure to glucose, oxidized LDL stimulates dendritic cells for maturation and attaining proinflammatory cytokine profile (Lu et al., 2013). These dendritic cells lose expression of LAP accompanied by CD86 and MHC-II upregulation with reduced expression of PDL1 (Yu et al., 2015). Conversely, supply of insulin reverses the inflammatory phenotype, as decreased RAGE expression, inhibition of PKCβ1, IRS1 serine and NF-κB phosphorylation were reported (Zhao et al., 2020).

3.3. Neutrophils

In this context, activity of the first-line responder, neutrophil, is also influenced in T1D. These immune system cells secrete various cytokines and chemokines, granules carrying enzymes, produce reactive oxygen species and neutrophil extracellular traps to fight with the infection. Under pathological conditions of T1D, the profile of neutrophils is readjusted, as microbicidal activity and adhesion decrease whereas controversial reports are present on some host defense mechanisms (Huang et al., 2016). For instance, the relation between high glucose in the environment and neutrophil adhesion is connected to increases in E-selectin, P-selectin and ICAM-1 levels on endothelial cells that promote endothelial cell-neutrophil adhesion in hyperglycemic conditions (Omi et al., 2002). In neutrophils, exposure to diabetic conditions lowers intracellular reactive oxygen species scavenger level and reshape transcription factors to control pro-inflammatory genes such as NF-κB and TGF-β. Hyperglycemia directs neutrophils to produce PKC activator and DAG, as well as increases NADPH oxidase levels resulting in oxidative stress and neutrophil extracellular trap formation. Glucose further alters characteristics of the extracellular environment, as hyperosmotic stress shrinks neutrophils and upregulates calcium influx. These mechanisms increase pro-inflammatory cytokines and can change phagocytic behavior. Moreover, AGE signals sensed by RAGE on neutrophil cell membranes provide persistence of inflammation. As a result, inflammation and tissue damage, and reduced bacterial removal occur in the diabetic body (Dowey et al., 2021).

3.4. Natural killer (NK) cells

Cytotoxicity of natural killer (NK) cells is crucial as body defense, but like many other cells, these cells are negatively regulated with unadjusted glucose dynamics in diabetes. In an effort to analyze these changes, a recent study established NK cells derived from T1D and T2D mice. Exposure of cancer cells to these cells led to the identification of reduced NK cell activity due to downregulation of activation markers such as NKG2D and NKp46, and apoptosis inducer, granzyme B compared to healthy state (Kim & Lee, 2022). NKp30/p46 and NKG2D levels are also decreased in T1D patients, yet the KIR2DS3 gene that codes the receptor on NK cells for HLA alleles is activated (Rodacki et al., 2007). In T2D, IFN-γ level as a representative factor of NK cell function decreased (Kim et al., 2019) and cell lysis activity of NK cells was lowered reversibly *in vitro* (Whalen, 2008).

3.5. T cells

T cells are another immune system family member that contributes to the development of T1D and suffers from pathobiology of it. These cells participate in different aspects of T1D onset and progression depending on the subset. Major effects of the transformed surrounding on T cells lie on for example, advanced glycation end products, since these products of glucose metabolism epigenetically modify T cells through p38 MAPK-dependent chromatin decondensation under continual tremendous amounts of glucose in the extracellular space. This allows transcriptional reprogramming and leads to higher levels of Th1, Th2, and Th17 cytokines in T cells (Martinez et al., 2014). Furthermore, stimulation of $CD4^+$ T cells by dendritic cells revealed that hyperglycemia decreases T cell proliferation and caspase-3 expression, but CD11a (an adhesion molecule for mobility) is enhanced resulting in improved T cell trafficking to the area of inflammation (Iwai et al., 2018). In contrast, high glucose favors T cell differentiation into Tregs through lactate driven glycolytic pathway rather than Th1 cells (Pitmon et al., 2023), yet a recent study put a new perspective that hyperlipidemia improves Akt-2 phosphorylation and thus, glycolysis is alleviated in Tregs. This results in higher frequency of IFN-γ- and IL-2-producing T cell number, but constant IL-17- and IL-4-producing T cells and, decreases FoxP3 and FoxO1 expression (Hyde et al., 2021). However, given that the lipid metabolism is dysfunctional, VLDL upregulated Th1 associated pathways in human and murine $CD4^+$ T cells *in vitro* and, higher CXCR3 and IFN-γ productions of T cells were evident in hyperlipidemic mice (van Os et al., 2023). Besides, it has been previously reported that patients with dyslipidemia have altered lymphocyte subset distribution (Xu et al., 2021). Complications of high glucose occur not only on the function and development

of T cells, but also susceptibility of these cells to infection is observed, as hyperglycemia induced CXCR4 and HIF-1α expression facilitate HIV entry into T cells (Lan et al., 2013).

3.6. B cells

In adaptive immune system, B cells are crucial for antibody-based response and to create memory. However, they are not shielded from adverse effects of hyperglycemia, as *in vitro* analysis indicated that secretion of immunoglobulin-M and its derivatives are lowered in B cells with high glucose treatment. These cells exhibited decreased proliferation accompanied with high apoptotic rate (Jennbacken et al., 2013). Although glycosylation is a mediator between activation and deactivation in the immune system in health and disease (Rudman et al., 2022; Wolfert & Boons, 2013), conformational changes due to nonenzymatic glycosylation of immunoglobulins in prolonged hyperglycemic conditions result in the decrease of complement fixation ending with lower amounts of microbe opsonization (Jafar, Edriss & Nugent, 2016). In a healthy body, leukocyte metabolism and function are correlated with insulin and its receptor on cell membrane and in diabetes, dysregulation and insulin resistance occur (Cruz-Pineda et al., 2022).

4. Islet non-cellular environment and its effects on beta-cells and immune system

4.1. Collagen

Collagens are triple helix of polypeptides (α-chains) with the functions ranging from tissue architecture to presynaptic organization (Ricard-Blum, 2011). Human pancreas is enriched by various collagen isoforms including fibrillar collagen I, II, III, V, XI, network-forming collagen IV, and collagen VI. Their expression is altered during the development as the adult matrisome contains higher amounts of, for example, collagen I, III, V, VI, VII than fetal pancreas (Hughes et al., 2006; Ma et al., 2019). α1 and α2 units of collagen IV are detected in the peri-islet membrane along with laminin chains α2, β1 and γ1, nidogen 1 and 2, and perlecan (Irving-Rodgers et al., 2008). Fibronectin, collagen I, III, VI, fibrilin-2 and matrilin-2 contribute to the NOD mouse pancreas interstitial matrix (Korpos et al., 2013).

Collagens are influential on various aspects of beta-cell biology such as collagen I being a regulator in beta-cell proliferation, viability and insulin secretion; collagen V in the improvement of the insulin secretion and organogenesis; collagen IV as a beneficial protein for increased beta-cell motility and insulin secretion, and collagen VI for islet survival and viability (Bi, Ye & Jin, 2020; Kaido et al., 2004; Krishnamurthy et al., 2008; Llacua et al., 2018a). Besides, degradation of collagens during islet

isolation for research or transplantation is a fundamental determinant for the consequences of the study (Meier et al., 2020). The recent findings show that these outcomes require differential activities of the collagen isoforms. For instance, integrin-β1/E-cadherin/β-catenin pathway is targeted distinctly by the collagen I and V resulting in promoting proliferation for collagen I and inhibition of this activity by collagen V (Zhu et al., 2021). To accomplish this, collagens interact with the integrins such as α1β1, α2β1, α3β1, αvβ1 of islets to signal through PI3K/Akt and MAPK/ERK pathways, regulate exocytotic protein localization as well as by binding discoidin domain receptors activating Pyk2-Rap1-(RPS6) axis (Barillaro, Schuurman & Wang, 2022; Llacua, Faas & de Vos, 2018b; Riopel & Wang, 2014).

In contrast, in T1D, the integrity of the basement membrane and the interstitial matrix of pancreas is compromised. In the onset, peri-islet capsule suffers from significant loss of ECM proteins especially at the sites of leukocyte infiltration which differs from the general strategy of leukocyte diapedesis from blood vessels (Korpos et al., 2013). For peri-islet membrane to be degraded, cell surface receptor modification and leukocyte transcytosis, MMPs and cathepsins are main enzymes to modify the extracellular space allowing islet infiltration by leukocytes (Korpos et al., 2013; Savinov & Strongin, 2009). This migration process of leukocytes in the body generally involves leukocyte LFA-1, selectins, α5β1 integrin, VLA4, CD44, CD99 and others to interact with endothelial layer. Eventually, enzymes such as neutrophil elastase and MMPs loosen the endothelial junctions so that leukocytes migrate through (Ley et al., 2007; Vestweber, 2015). In chronic diseases such as rheumatoid arthritis and chronic lung diseases, cleaved hyaluronan fragments and others activate infiltrating leukocytes through TLR extending the inflammation (Sorokin, 2010). Importantly, the $CD8^+$ T cells residing in the healthy exocrine pancreas are specific for the preproinsulin (Bender et al., 2020) and β1 integrin mediated T cell motility guided by exocrine pancreas ECM is also required for immune cell accumulation and activity in the pancreas. It highlights β1 signaling axis as a potential target to promote new therapies (Espinosa-Carrasco et al., 2018b). These data indicate that the abnormal activity of the exocrine pancreas is partly responsible for T1D. Another aspect on the changes in the collagen I abundance proved that collagen layer is fragmented in T1D and destruction-resynthesis mechanism is followed during disease progression (Wang et al., 2019).

4.2. Laminin

Laminins are a family of trimeric glycoproteins with α, β and γ chains which are combined with cell/tissue-specificity. This subgroup is involved

in pancreatic organogenesis and cell differentiation (Virtanen et al., 2008). Laminin chain isoforms are distinctly distributed in the pancreas and within the islets, distribution of laminin is present around the blood vessels. For instance, laminin α4 and laminin β2 are limited to intra-islet blood vessels and exocrine tissue, but no presence on the mouse islet surface coating (Vigier et al., 2017). In NOD mice peri-islet basement membrane, laminin α2, α4, β1, β2, γ1, γ2, and γ3 chains but not laminin α1, α3, and α5 chains are reported. By contrast, basement membranes in the pancreas blood vessel are positive for laminin α4 and α5 chains together with laminin γ1 and β1 chains (Korpos et al., 2013). As such abundant proteins in the pancreas, laminins improve insulin secretion from human islets in glucose-stimulated insulin secretion test (Hadavi et al., 2019) and in laminin supplemented gels, single beta-cells display less apoptosis and higher insulin secretion (Weber, Hayda & Anseth, 2008). These trimetric glycoproteins are useful to ensure the control on the adhesion (Virtanen et al., 2008) and the expression of islet specific genes such as Pdx-1, insulin 1, insulin 2, glucagon, somatostatin and glucose transporter-2 (Llacua, et al., 2018a). Laminin 511 isoform has been implicated on its ability to aid beta-cell proliferation and partial inhibition of epithelial-to-mesenchymal transition of beta-cells (Banerjee et al., 2012). This group of proteins shows activity towards upregulation of FGFR5 that heterodimerizes with FGFR1. This signaling activates expression of maturity marker UCN3 and rate-limiting enzymes of glycolysis (PKM2, GCK) to increase glucose metabolism (Pal et al., 2022). To do so, laminins bind to integrins such as α3β1 or non-integrin receptors of Lutheran group glycoprotein (Virtanen et al., 2008), α-dystrogycan (Jiang, Georges-Labouesse & Harrison, 2001) and 67kDa laminin receptor (Sabra et al., 2015) of pancreatic islets/beta-cells.

In the immune system, laminins mediate monocyte to macrophage differentiation (Li, L. et al., 2020), harnessing tolerance or immunity in lymph nodes (Warren et al., 2014), adhesion, migration, activation and function of innate and adaptive immune system cells (Simon & Bromberg, 2017) pointing out its possible contribution to beta-cell destruction.

4.3. Fibronectin

As a glycoprotein formed of a disulfide bond linked dimer, fibronectin harbors binding sites for collagen, heparin, fibrin, glycosaminoglycans and proteoglycans; integrin receptors such as α5β1; growth factors including TGF-β, FGF, PDGF, HGF and VEGF; and bacteria. This allows cell-matrix interactions, directing host-microbe interactions, regulating availability of growth factors for angiogenesis, proliferation and many other vital processes of the cells (Dalton & Lemmon, 2021). It is also tract for T cells in the inflamed skin (Fernandes et al., 2020). As one of the major

components of the islet ECM, fibronectin binding to αvβ1, αvβ3 or α5β1 integrin of beta-cells is favorable for the proper functioning of beta-cells (Weber et al., 2008). Transplantation of fibronectin treated islets into the kidney capsule of streptozotocin-treated rats revealed better glucose tolerance compared to untreated control group reflecting the importance of ECM for the success of islet transplantation outcome. Through this action, higher islet cell mass, better viability with preserved function are beneficial consequences of the ECM proteins such as fibronectin (Hamamoto et al., 2003). Moreover, combination of the cues from the fibronectin as well as other ECM proteins can have prominent effects. In one of such studies, fibronectin mimic motif (GGRGDSP) with the collagen IV mimic motif (GEFYFDLRLKGDK) containing self-assembling peptide drove assembly of islet-like aggregation of INS-1 insulin secreting cells as well as promoted ECM (fibronectin, collagen IV), cell-cell adhesion (E-cadherin) and beta-cell function (Glut2, Ins1, MafA, Pdx-1) gene expression. Within this system, proliferation of beta-cells was noticeable via integrin α5/β1-FAK-ERK-cyclin D1/p27 pathway (Liu et al., 2015).

4.4. Nidogen

Nidogen (entactin) is a glycoprotein with two isoforms (nidogen-1 and nidogen-2) in human and mouse. Both isoforms comprise three globular domains, one linker between G1 and G2 domains as well as a rod segment between G2 and G3. The classification of nidogen-1 and nidogen-2 is based on the differences in the rod segments and the G3 domains. The rod segment of nidogen-2 consists of four EGF-like modules and two thyroglobulin type-1 modules whereas nidogen-1 is formed with four EGF-like modules and one thyroglobulin type-1 module. In the globular domain G3, both isoforms have six low density lipoprotein receptor YWTD repeat and, nidogen-1 has one extra EGF-like module (Ho et al., 2008). Nidogens cooperate with the laminin through their G3 domains and additionally, G2 domain of the nidogen-1 can interact with collagen IV and perlecan. Therefore, nidogens act a bridge to connect collagen IV and laminin networks in the basement membrane. Nidogen functions in the body include cell fate determination and angiogenesis. A potential integrin interaction site as an RGD motif is accounted in the EGF-like repeat of the rod domain in nidogen-1 and in the last EGF-like repeat in murine nidogen-2 (Zhou et al., 2022). Nidogen is also abundant in peri-islet ECM that is synthesized and managed by vascular cells and peri-islet fibroblasts. During islet isolation, islets lose peri-islet basement membrane and thus, their function is diminished. Therefore, laminin, nidogen and collagen IV protein-based assembly of islet basement membrane rescues islets from the negative effects of isolation (Santini-González et al., 2021). Moreover, nidogen-1 treatment of human EndoC-βH3 cell line pseudoislets improved insulin secretion and

E-cadherin expression, and protected beta cells against ischemia-induced death due to αvβ3 linked activation of MAPK pathway. Under hypoxic conditions, mitochondrial function, insulin, and insulin-transporting lipid vesicles expression increases were noted as the underlying mechanism of higher insulin secretion compared to control (Zbinden et al., 2021). In immune system, nidogen 1 manipulates polarization of macrophages, as treatment with this protein directed macrophage fate to M0 or M2 phenotype rather than pro-inflammatory one, yet it is an activator for $CD4^+$ T cells (Zbinden et al., 2021).

4.5. Heparan sulfate proteoglycans (HSPGs)

Heparan sulfate proteoglycans (HSPGs) have core proteins covalently linked with repeating disaccharide units of glucosamine and uronic acid. Negative charged HS chains allow binding of various proteins including pro-angiogenic growth factors, enzymes and enzyme inhibitors, cytokines and cell adhesion molecules. Thus, they fulfill diverse tasks in the angiogenesis, inflammation, cancer, lipid metabolism and basement membrane establishment (Bishop,Schuksz & Esko, 2007). Highly expressed by alpha-cells and contributed by beta-cells, HSPGs are fundamental constituents of ECM which rescues beta-cells from the effects of oxidative stress (Theodoraki et al., 2015). These group has extracellular and transmembrane members, and transmembrane syndecan-4 of MIN6 beta-cells is observed to positively regulate glucose stimulated insulin secretion (Takahashi, Yamada & Nata, 2018). The core proteins of HSPGs such as Col18, syndecan-1 and CD44 isoforms are also intracellularly produced by beta-cells parallel to intra-islet HS production (Choong et al., 2015). As the islet isolation progresses for the purpose of research or transplantation, lost HS is not recovered during culture or when supplemented with HS-mimetics, but significant improvement is evident in the isotransplantation with the effect of *de novo* synthesis (Choong et al., 2015).

Heparanase highly expressed by activated endothelial cells and islet-infiltrating mononuclear cells in diabetes cleaves HS and triggers burst release of cytokines such as TNF-α, IFN-γ and ILs leading to beta-cell death. Therefore, simultaneous treatment of human islets with heparanase and HS-mimicking glycopolymer downregulates IL8, IL-1β, TNFα and TLR2 expressions (Loka et al., 2022). In another study, OGT2115 (a heparanase inhibitor) decreased beta-cell loss and infiltration of mononuclear macrophages, $CD4^+$ and $CD8^+$ T cells shielding beta-cells from immune attack (Song et al., 2020). Alpha cells also contain HS domains, but sulfation status and abundance differ from beta cells. In alpha cells, HS domains have abundant 2-O, N-sulphated and non-sulphated sequences, low in 6-O-sulphation and C5-epimerisation. Beta cells have 2-O, 6-O and

N-sulphation, and C5-epimerisation (Theodoraki et al., 2015). However, HS side chains can bind to IAPP secreted by beta-cells altering the IAPP conformation and facilitating extracellular fibril deposition, therefore amyloid toxicity and probably beta-cell loss in T2D (Oskarsson et al., 2015).

4.6. Hyaluronic acid (HA)

Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan of repeated disaccharides units of (β1→4)-glucuronic acid and (β1→3)-N-acetylglucosamine synthesized by transmembrane hyaluronan synthases. It takes great attention with its benefits on the skin, eyes, joints and intestine health (Zheng et al., 2023). To do so, HA interacts with CD44, RHAMM, stabilin2, LYVE-1, layilin and TLR4 of a plethora of cells for signaling, HA clearance from circulation, inflammasome activation, wound healing, migration, proliferation and many other physiological processes (Garantziotis & Savani, 2019). As a part of the peri-islet and intra-islet (around islet microvessels) ECM, hyaluronic acid is synthesized by hyaluronan synthases 1 and 3, and its binding partners (tumor necrosis factor–stimulated gene 6, inter-α-trypsin inhibitor and bikunin) are expressed differentially in the endocrine cells of the pancreas (Bogdani et al., 2014; Hull et al., 2012).

This polysaccharide and its binding partners (inter-α-inhibitor and versican) show considerable accumulation in the human islets during the development of T1D. They surround inflammatory cells and even tumor necrosis factor–stimulated gene 6 is expressed in these set of cells. Excess HA is also present in the lymphoid tissues. This data demonstrates the contribution of HA and its binding partners to the inflammatory driving mechanism of T1D (Bogdani et al., 2014; Hull et al., 2015). Likewise, Treg differentiation is inhibited by HA, therefore exerting potential participation to the impaired tolerance in T1D (Nagy et al., 2015). In line with this finding, HA receptor, CD44, expression induces beta-cell death as well as reduces insulin content and glucose stimulated insulin release (Assayag-Asherie et al., 2015). A separate mechanism of HA further manipulates antigen presenting cells, since requirement of CD44:HA:LYVE-1 axis is defined for dendritic cell migration into the lymph nodes (Johnson et al., 2021). Interestingly, human amniotic epithelial cells differentiate into insulin producing cells in the presence of HA and that activity is shown to be promising to reach normoglycemia *in vivo* (Luo et al., 2019). Furthermore, beta-cell culture on HA-coated surfaces enhances insulin secretion of hamster HIT-T15 cells by improving connexin 43-mediated communication (Li, Nagira & Tsuchiya, 2006) and rat Ins1E cells microencapsulated in alginate-HA gel has better viability with no significant effect on the insulin secretion (Cañibano-Hernández et al., 2019). Collectively, these results indicate that a complex interaction network of HA with islets and presence

of immune system determines the precise outcome of the alteration in HA *in vitro* and *in vivo*.

5. Conclusion

Complexity of T1D manifests itself on the cells and extracellular matrix. Although many driving factors for T1D are discovered, main players remain as innate and adaptive immune system. Disturbed nature of immune system cells promotes further beta-cell destruction which eventually causes significant loss of insulin secretion and sustains considerable level of glucose in circulation. Onset and progression of T1D are driven by these cells and in turn, complications associated with hyperglycemia affect immune system. In between, ECM is also manipulated to allow destruction or to support beta-cell cells shaping the course of T1D. The literature proves that noncellular microenvironment is vital for healthy and diseased state, and its contribution should not be underestimated. In T1D, beta-cell, immune system and extracellular matrix circle should be revisited to improve the clinical outcome of the T1D treatment.

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CHAPTER 6

FUNDAMENTALS OF PET/CT FOR CLINICIANS

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Introduction

Positron emission tomography (PET) is a three-dimensional scintigraphic imaging process using positron-emitting radionuclides. Fluorine (F)-18, Carbon (C)-11, Oxygen (O)-15, Nitrogen(N)-13 and Gallium (Ga)-68 are the most commonly used positron-emitting radionuclides. Positron-emitting radionuclides are generally elements with low atomic numbers and very short half-lives. They can be produced artificially in closed particle accelerator systems called cyclotrons.

Positrons emitted as a result of the decay of these radionuclides collide with the electrons they encounter. As a result of the collision of positrons with electrons, the mass of both particles is converted into energy and annihilated. This phenomenon is called annihilation. As a result of the annihilation event, two gamma photons are formed at a constant energy of 511 keV in 180° opposite directions. This high-energy photon pair is detected by PET detectors in PET scanners capable of "coincident (simultaneous) detection" and converted into images.

PET imaging can image various biochemical events in the organism in vivo by using radiopharmaceuticals obtained by binding positron-emitting radionuclides with different molecules. The biggest advantage of PET imaging over radiological imaging methods is that it can show functional and metabolic changes in the early stages of many pathological conditions where structural disorders have not yet occurred.

PET imaging is based on the "emission" principle, like other nuclear medicine methods and magnetic resonance imaging. Due to the distribution of radiopharmaceuticals to different tissues within the body, photons formed as a result of annihilation pass through different tissue layers at different densities while leaving the body. This causes different photons to lose energy at different rates depending on the tissue types they pass through. This is called attenuation. In PET devices, in addition to emission imaging created by annihilation photons coming from the patient's body, transmission imaging is also performed for attenuation correction. Thanks to the attenuation correction made with transmission imaging, a more accurate measurement of the radioactivity concentration per unit area in emission images is possible and semiquantitative parameters such as standardized uptake value (SUV) expressing this can be calculated. The most frequently used method in clinical use is standardized uptake value (SUV), which is a semiquantitative evaluation method. It shows the radioactivity concentration in the selected area. If the injected radiopharmaceutical was distributed homogeneously throughout the body, the measured SUV value would be 1.

SUVmax gives the highest SUV value in the measured area. SUVmax measurement is the most frequently used method because it is easy,

repeatable and widely available in software programs used in clinics. SUV, which is a semiquantitative parameter, can be affected by factors such as the patient's plasma glucose level, the time between the injection and the start of imaging, body weight, and the features of the scanner.

For SUV measurement, a 2-dimensional region of interest (ROI) or 3-dimensional volumetric region of interest (VOI) is drawn on PET images via workstations. The highest SUV value of the lesion is called SUVmax. Although the mean SUV (SUVmean) is highly dependent on the plotted VOI area, the SUVmax value is largely reader-independent. SUVmax is used for prognosis and treatment evaluation in many types of cancer.

Positron emission is known as beta-positive decay, which is an isobaric decay, and as the proton in the radionuclide nucleus turns into neutrons, positrons and neutrinos are released. This decay is seen in proton-rich radionuclides and results in the formation of an isobaric element with one less atomic number but the same mass number. The positron is the antiparticle of the electron. During its course, the positron combines with an electron and annihilation occurs. As a result of this event, two gamma-ray photons are formed, each with an energy of 511 keV, and they are emitted in approximately 180 degrees opposite directions from each other. These photons are detected by electronic collimation by mutual PET detectors. The arrival of annihilation photons reaching the opposite detectors is recorded in a very limited time at the level of 3-15 nanoseconds, and the detection of both photons in this narrow time interval forms the basis of coincidant detection, one of the basic principles of PET. It is assumed that the location of the annihilation photon occurs somewhere on the straight line drawn on two opposite detectors called the line of response (LOR).

Since positron-emitting radionuclides are highly biogenic elements, they can easily bind to biomolecules and thus offer great potential for in vivo imaging of body chemistry by creating effective radiopharmaceuticals for the desired biochemical event. In addition, since the simultaneous detection technology in PET scanners provides better resolution and less scattering, the image quality obtained is significantly superior to scintigraphic images obtained with single photon emitters in conventional gamma cameras.

PET/CT is an advanced technology imaging method used in many fields such as oncology, cardiology and neurology. In PET imaging, pharmaceuticals are labeled with positron-emitting radioisotopes such as F-18. In PET imaging, hundreds of radiopharmaceuticals are available that can monitor many biochemical and physiopathological processes ongoing in various organ systems in the living organism.

F-18 FDG PET/CT

F-18 FDG PET/CT is a hybrid imaging method that allows us to obtain quantitative parameters that show the metabolic activity of target tissues. The most commonly used PET radionuclide is F-18, a cyclotron product. It is obtained by bombarding water enriched with O-18 by high-energy protons. Its half-life is 109.7 minutes, it emits 97% beta particles and its maximum energy is 0.64 MeV. Low positron energy causes its range in tissue to be short (2.3 mm), thus providing slightly higher resolution and lower radiation dose exposure. This is one of the reasons why F-18 is the most commonly used PET radionuclide.

The most common pharmaceutical labeled F-18 on PET imaging is fluorodeoxyglucose (FDG). FDG is a glucose analog and allows us to evaluate the glucose metabolism of tissues. Increased anaerobic glycolysis in malignant cells causes an increase in FDG uptake. In addition, the increase in glucose carrier proteins (especially GLUT-1) in the membrane of these cells and the hexokinase enzyme that phosphorylates inside the cell also causes an increase in FDG uptake. FDG is taken into the cell in the same way as glucose, through cell surface transport proteins such as GLUT-1 and GLUT-3, but their intracellular metabolism occurs differently. Once both glucose and FDG enter the cell, they are phosphorylated by hexokinase in most cells. After phosphorylation, they cannot leave the cell due to their negative charge. After this stage, glucose-6-phosphate is dephosphorylated by glucose-6-phosphatase and metabolic processes continue by undergoing glycolysis. Since FDG-6-phosphate is missing a hydroxyl group at the C-2 position, it cannot be metabolized further and is trapped intracellularly. This is the most important difference between native glucose and FDG and provides the advantage of imaging. In addition, low glucose-6-phosphatase enzyme activity in malignant cells prevents destruction by reducing dephosphorylation. This leads to FDG accumulation in cells. Positrons released into the environment as a result of the degradation of the Fluorine-18 component of FDG accumulated in the cell collide with electrons and create annihilation photons. Thanks to the emission images created by detecting these photons, the differentiation of tissues in glucose metabolism is evaluated. However, increased glucose metabolism in cells does not always indicate malignancy. Increased FDG uptake can also be observed in inflammatory-infectious events due to the increase in glucose metabolism. Higher glucose transport protein expression, increased cell proliferation, protein and DNA synthesis, and excessive neoangiogenesis are generally observed in cancer cells. The use of glucose to produce energy through anaerobic glycolysis has increased to meet the increased metabolism due to the increased cell division and associated hypoxia observed in cancer cells. In F-18 FDG PET/CT, which is a hybrid imaging tool, low-dose CT

imaging methods are used together with PET. While the patient is in the same bed position, PET and CT images are taken sequentially and combined. With CT, both anatomical correlations of the lesions observed in PET images can be made and attenuation correction can be made.

Patient preparation for FDG PET/CT imaging is of utmost importance. To summarize patient preparation:

• According to the European Association of Nuclear Medicine (EANM) guidelines, the patient's blood glucose level should not be above 200 mg/dl before imaging.

• The patient must fast for at least 4 hours before imaging. However, he can drink water during this period.

• Parenteral nutrition or IV fluids containing dextrose should be discontinued at least 4 hours before imaging.

• Consuming 1 liter of water, starting before imaging, is recommended to reduce background activity and bladder radiation dose.

• Recommended practices for Type 1 DM and insulin-dependent Type II DM patients can be listed as follows. In patients with a continuous insulin infusion pump, insulin infusion should be stopped at least 4 hours before FDG injection. These patients should have breakfast after imaging and continue insulin infusion after breakfast. In patients using medium-long acting insulin preparations, after the evening insulin injection, fasting overnight and then imaging should be performed in the morning. After the imaging, the patient can have breakfast. In patients using short-acting insulin preparations, after the patient has breakfast early in the morning and takes the insulin injection, imaging should be performed at noon, at least 4 hours after the short-acting insulin injection, and at least 6 hours after the rapid-acting insulin injection.

• In patients with Type II DM, which can be controlled with oral antidiabetic treatment, imaging can be performed by observing the fasting rule of at least 4 hours, which normal patients must follow, while the patient continues oral antidiabetic treatment. However, it should be taken into consideration that metformin treatment may increase colonic FDG uptake. It is recommended that sulfonylurea group oral antidiabetic drugs should not be taken in the morning of the imaging day. There is no need to discontinue oral antidiabetic drugs such as rosiglitazone and pioglitazone.

• If short-acting insulin injection was made intravenously to lower blood sugar before imaging, FDG injection can be done 30-90 minutes later. However, if a rapid-acting insulin injection was made subcutaneously to lower blood sugar before imaging, FDG injection should be done at least

4 hours later.

• The patient should avoid strenuous exercise for at least 24 hours before imaging.

• The patient should not talk 5 minutes before and 20 minutes after FDG injection. Otherwise, laryngeal muscle involvement may be observed.

• Particularly in cold weather, brown adipose tissue involvement may occur due to adrenergic stimulation, making evaluation difficult. For this reason, the patient should not be exposed to cold if possible before imaging. It is recommended that patients avoid exposure to cold and dress warmly, if possible, for at least 48 hours before imaging.

• It is recommended that patients undergo PET/CT imaging at the earliest 1 week after biopsy procedures.

• According to the European Association of Nuclear Medicine (EANM) guidelines, patients must have a PET/CT scan at least 6 weeks after the surgery.

• Patients must have a PET/CT scan at least 4 weeks after previous radiofrequency ablation treatment.

• Patients must have a PET/CT scan at least 10-14 days after chemotherapy.

• Patients must have a PET/CT scan at least 2-3 months after radiotherapy.

• Patients should have a PET/CT scan at least 5 days to 1 month after granulocyte colony-stimulating factor treatment.

• In breastfeeding mothers, contact between mother and child should be stopped until 12 hours after FDG injection. During this period, milk can be expressed and given to the child.

To give brief information about FDG PET/CT indications;

- FDG PET/CT can be used to help prevent an invasive diagnostic procedure for diagnosis/metabolic characterization in masses suspicious of malignancy or to guide an invasive diagnostic procedure.

- It can be used for staging purposes in patients diagnosed with cancer to determine the extent of the disease. To perform PET for staging purposes, the patient must not have started chemotherapy and/or radiotherapy treatments. In these patients, PET/CT imaging can also be performed for radiotherapy planning purposes.

- If any relapse/recurrence is detected during the follow-up phase of cancer after the initial treatment or if there are findings in favor of re-

currence, PET/CT imaging may be performed for restaging purposes to investigate whether there are other metastases or to show the extent of the disease.

- After completion of chemotherapy, radiotherapy or immunotherapy, PET/CT imaging can be performed for treatment response evaluation to investigate the response of the tumor.

- In cancers that are treated only with chemotherapy and alternative chemotherapy protocols can be applied, PET/CT imaging can be performed to determine chemosensitivity to investigate the response of the tumor to the applied chemotherapy protocol in the early period (after the 1st-3rd cycle). These imaging are called interim PET.

In oncological PET studies, the scan covers the distance from the skull base to the upper thighs. Since PET has low sensitivity in demonstrating brain metastases, it is not necessary to include the cranial region in imaging in standard oncological PET studies. In malignancies such as malignant melanoma, neuroblastoma and multiple myeloma, which have a very high potential for metastasis, the cranial region and lower extremities are included in whole-body imaging.

"European Organization for Research and Treatment of Cancer (EORTC)" and "PET response criteria in solid tumors (PERCIST)" criteria are used to evaluate treatment response. According to these criteria, patients are classified into 4 categories: complete metabolic response (CMR), partial metabolic response (PMR), stable metabolic disease (SMD), and progressive metabolic disease (PMD). While the first of these criteria, EORTC, uses the SUVmean value as a quantitative parameter, the PERCIST criteria use the (SUL) value obtained by correcting the SUVpeak value according to lean body mass (LBM).

According to EORTC, "loss of FDG uptake (indistinguishable from surrounding normal tissue)" is used as the criterion for a complete metabolic response. According to EORTC, the criterion for partial metabolic response is "15-25% decrease in SUV after one cycle of chemotherapy, <25% decrease in SUV after more than one cycle of chemotherapy". According to EORTC, the criterion for stable metabolic disease is "SUV < 25% increase or < 15% decrease and no significant increase in the prevalence of FDG tumor involvement (< 20% increase in long diameter)". According to EORTC, the criterion for progressive metabolic disease is "SUV > 25% increase or 20% increase in long diameter or new FDG-enhancing lesion(s)".

According to PERCIST, "FDG uptake cannot be distinguished from surrounding background activity and SUL < liver and no new lesions oc-

cur" are used as criteria for a complete metabolic response. According to PERCIST, the criterion for partial metabolic response is "≥ 30% decrease in SUL, at least 0.8 unit difference in the target lesion and no new FDG uptake lesions and < 30% size increase in the target lesion and no SUL or size increase in non-target lesions." and no new lesion formation". According to PERCIST, the criterion for a stable metabolic disease is "There should be no more than 30% increase or decrease in SUL and does not fit into the PMD, PMR and CMR categories". According to PERCIST, the criterion for the progressive metabolic disease is "at least a 30% increase in SUL, at least a 0.8 unit increase in the target lesion or at least one newly developed lesion, or a 30% increase in the target lesion size or progression in non-target lesions." is used.

Today, staging and treatment response evaluation of lymphomas are performed with FDG PET/CT. Deauville Scoring (DS) is used for this purpose. With the five-step DS, evaluation is made according to the mediastinal blood pool and liver activity. According to this scoring, score 1 is "No FDG uptake", score 2 is "FDG uptake level is equal to or less than the mediastinum", score 3 is "Uptake more than the mediastinum but less than the liver", score 4 is "Moderately increased FDG uptake in any region compared to the liver", score 5 is classified as "Significantly increased FDG uptake in any area or new areas of disease" and the score "X" is classified as "New areas of FDG uptake thought to be unrelated to lymphoma". In post-treatment and interim evaluations, the classification for score 1 and score 2 is the same, but it is different for score 3, score 4 and score 5. In interim PET evaluation, score 4 and score 5 with decreasing FDG uptake compared to the initial imaging are classified as partial response, and score 4 and score 5 with increased FDG uptake are classified as progressive disease. In the post-treatment evaluation, scores 4 and score 5 are considered treatment failure. In post-treatment and interim evaluation, score 3 is considered negative. In a patient who shows a good response to treatment in known disease areas in the Lugano classification, if an FDG-enhancing lesion is detected in another area after treatment, it is evaluated as Deauville X. Although there is more FDG uptake in the Waldeyer ring-intestine-chemotherapy and granulocyte colony-stimulating factor (G-SCF)-activated spleen and bone marrow, where FDG uptake is physiologically intense, than in the normal liver, it is considered as a complete metabolic response. If the primary disease is in these areas, in the evaluation of treatment response, the activity level that does not exceed the surrounding normal tissue in the same lesion area is considered normal and evaluated as a full metabolic response. In lymphomas with little or no FDG uptake, such as marginal zone, small lymphocytic type lymphomas and some cutaneous lymphomas, follow-up is performed with CT.

Ga-68 PSMA PET/CT

Prostate cancer is one of the most common malignancies in men, although it varies around the world. Prostate cancer is the fifth most common cause of death worldwide. Prostate Specific Antigen (PSA) value, Gleason score (GS) and tumor stage are taken into consideration when planning the prognostic classification and treatment management of prostate cancer patients. Prostate-specific membrane antigen (PSMA) glutamate carboxypeptidase II is an internal transmembrane glycoprotein that was first identified in 1987. PSMA is a type II transmembrane glycoprotein consisting of 750 amino acids, known as glutamate carboxypeptidase 2 or N-acetyl-L-aspartyl-L-glutamate peptidase, with folate hydrolase activity, expressed by the prostatic epithelium. While PSMA is expressed at low levels in normal prostate tissue and benign pathologies, it is 100 to 1000 times more intense in prostate cancer and increases with increased tumor aggressiveness - intratumoral angiogenesis. Ga-68 is a Ge-68/Ga-68 generator product with 89% positron emission and a half-life of 67.63 minutes. Ga-68 PSMA is physiologically retained in the lacrimal-submandibular-sublingual and parotid glands, and also shows normal biodistribution in the liver, spleen, small intestine, colon and kidneys. Although the radiopharmaceutical is primarily excreted in the kidneys, there is also partial hepatobiliary clearance. Additionally, celiac ganglia show low levels of Ga-68 PSMA uptake. Ga-68 PSMA PET/CT is an imaging method with high sensitivity in the detection of primary tumors and metastases and in detecting recurrence, so it is increasingly used in primary staging and restaging. PSMA expression levels increase with stage and tumor grade, as well as in the case of the development of castration resistance. The most important advantage of Ga-68 PSMA is that it is superior to F-18 choline and other currently FDA-approved agents (C-11 choline, F-18 Fluciclovine) on PET imaging at low PSA values in detecting PC recurrence (average sensitivity 76%-86, specificity 86-100%). If we summarize the indications for the use of Ga-68 PSMA PET/CT examination in prostate cancer patients, they can be listed as staging, detection of recurrence area in cases of biochemical recurrence, and evaluation of response to treatment. It is not recommended to use PSMA PET/CT for diagnostic purposes. Ga-68 PSMA PET/CT can also be used for RT planning purposes.

To summarize the patient preparation steps for Ga-68 PSMA PET/CT imaging:

• Patients do not need to fast.

• Patients can continue to use all the medications they have used.

• Patients should consume 500 ml of water within 2 hours before imaging.

• Immediately before imaging, the patient should be allowed to empty his/her bladder by urinating.

• "Furosemide" injection can be performed during imaging to reduce urinary activity in patients with no medical contraindications.

Ga-68 DOTA-TATE PET/BT

Somatostatin is a peptide that is released from many different tissues. In general, it has an inhibitory effect on hormonal systems. While it reduces the release of hormones such as growth hormone, insulin, glucagon, gastrin and serotonin, it also has antiproliferative activity on tumors. Somatostatin exerts its effects through its receptors located on the cell surface. Six types of somatostatin membrane receptors have been identified in the human body: SSTR1, SSTR2A, SSTR2B, SSTR3, SSTR4 and SSTR5. SSTR2 is the type most expressed in tumors and normal tissues. Neuroendocrine tumors (NET) are one of the rare types of cancer, but one that has shown an increasing trend in recent years.

Neuroendocrine tumors (NET) express somatostatin receptors (sst-r) at rates reaching 80%. While it is especially found in sst-r type 2 tumors, it is expressed less in tissues other than the pituitary. While somatostatin receptors are higher in low-grade tumors, they decrease inversely with increasing grade. 68Ga-DOTA-TATE, 68Ga-DOTA-TOC, and 68 Ga-DOTA-NOC are the most commonly used PET radiopharmaceuticals for somatostatin receptor scintigraphy. It is a Ga-68 generator product (Germanium-68/Galium-68 generator). Therefore it is easy to obtain. Ge-68, the main radionuclide of the generator, has a half-life of 270.95 days. The generator allows Ga-68 to be obtained practically for a period of approximately 9-12 months due to its relatively long half-life of the parent radionuclide. Thus, after purchasing the generator, it is possible to easily prepare radiopharmaceuticals such as 68Ga-DOTA-TATE in any nuclear medicine department with suitable laboratory conditions.

In the European Association of Nuclear Medicine guideline (EANM), Ga-68 DOTA-peptide PET/CT is used for staging, follow-up and restaging of NETs in relapsed patients, to investigate the primary focus when NET metastasis of unknown primary is detected, to identify the primary/recurrent focus at high levels of specific tumor markers. It is recommended to be used in the evaluation of bronchial masses suggestive of NET for investigation purposes. It can also be used to detect SST receptor expression of tumors to decide on PRRT or long-acting somatostatin analog treatment.

Investigation of residual disease after resection and evaluation of the response to systemic treatments are also among the indications for Ga-68 DOTA-peptide PET/CT. However, since the probability of metastasis is

very low in "gastric neuroendocrine tumors (Type I) that develop based on atrophic gastritis, in incidentally detected <2 cm R0 appendiceal NETs, and <1 cm R0 (T1N0) incidentally detected rectal NETs that can be subjected to R0 resection" the use of Ga-68 DOTATATE PET/BT is not recommended. Ga-68 DOTA-peptides can also be used to detect the focus in the diagnosis of ectopic Cushing syndrome.

To summarize the patient preparation steps for Ga-68 DOTA-TATE PET/CT imaging:

• Patients do not need to fast.

• For somatostatin receptor antagonist treatments used by patients, it is recommended that short-acting ones be discontinued 1 day before imaging and long-acting ones 3-4 weeks before imaging, unless contraindicated.

• Immediately before imaging, the patient should be allowed to empty his/her bladder by urinating.

• In case of breastfeeding: if imaging is necessary, breastfeeding should be interrupted for 7 physical half-life periods (approximately 7 hours).

Brain PET/CT

PET imaging is a functional imaging technique that finds wide application in neurological diseases. With this technique, the pathophysiology of neurodegenerative diseases can be investigated with three-dimensional cross-sectional imaging by using many radioactive biological compounds that can be retained in brain tissue. PET images obtained by labeling a metabolically active compound with a radioactive isotope are combined with the computerized tomography (CT) technique and also provide information about the anatomical details of the brain. The most commonly used PET agent in the clinic is FDG (F18-Fluordeoxyglucose).

The most common areas of use are epilepsy, dementia and brain tumors. Similar to SPECT in epilepsy, it shows the epilepsy focus as hypometabolic in the interictal period. It shows hypometabolic areas in the same regions according to etiology as SPECT in dementia. Due to high physiological glucose metabolism in normal brain tissue, FDG uptake appears intensely in the gray matter, but uptake is weak in the white matter. If brain PET/CT imaging indications are to be listed, they can be divided into 4 main groups: dementia disorders, neuro-oncology, epilepsy and movement disorders.

It is an imaging method that helps in differential diagnosis among dementia diseases. It can be used in the differential diagnosis of space-occupying formations in the brain and in detecting the presence of recurrent tumors. Locating the epileptic focus is an important indication of FDG

PET/CT. It can be used in the differential diagnosis of Parkinson's disease and atypical Parkinsonian syndromes.

To summarize the patient preparation steps for brain FDG PET/CT imaging:

• According to the European Association of Nuclear Medicine (EANM) guidelines, the patient's blood glucose level should not be above 160 mg/dl before imaging.

• The patient must fast for at least 4 hours before imaging. However, he can drink water during this period.

• Intravenous dextrose-containing or parenteral nutrition fluids should not be administered for 4-6 hours before imaging.

• The patient must wait in a quiet, dimly lit room before and for 20 minutes after the injection.

• The patient must remain motionless for at least 30 minutes before FDG injection and during the uptake phase (at least 30 minutes) after the injection.

• The patient should not talk, engage in physical activities or read anything after the injection.

• Immediately before imaging, the patient should be allowed to empty his/her bladder by urinating.

• If PET is indicated for breastfeeding mothers, they should take a break from breastfeeding for 24 hours.

• The use of caffeine, alcohol and drugs that may affect brain glucose metabolism should be avoided.

• Continuous EEG monitoring is recommended in patients who will undergo preoperative evaluation before epilepsy. To ensure that the FDG injection is not administered in the post-ictal phase, this EEG monitoring should ideally begin 2 hours before injection and continue for at least 20 minutes after injection.

Ga-68 FAPI PET/CT:

Another PET imaging modality that is gaining importance today is FAPI-PET. FAPI is a surface integral membrane protein expressed from cancer-associated fibroblasts (CAF). It is a PET agent that is predicted to contribute to primary diagnosis, detection of metastases and treatment by marking FAP proteins on the surface of cancer-related fibroblasts with FAP inhibitor molecules. It was labeled with the radioactive element Ga-68 in a peptide-mediated manner and started to be used in PET/CT imaging.

It provides tumor imaging with high sensitivity in many types of cancer, especially epithelial cancers and cancers with high desmoplasia content. In this imaging method, the time between injection and imaging is much shorter since tumor activity uptake is faster than FDG. In addition, the tumor/background ratio is better with Ga-68 FAPI due to the absence of physiological activity in tissues such as the brain, heart and liver.

To summarize the patient preparation steps for Ga-68 FAPI PET/CT imaging:

• Patients do not need to fast.

• It is not necessary for the patient's blood sugar level to be at a certain level.

• Immediately before imaging, the patient should be allowed to empty his/her bladder by urinating.

Myocardial PET/CT

Differentiating viable or nonviable myocardial tissue in patients with coronary artery disease and left ventricular dysfunction is critical for treatment management. Improvement of left ventricular dysfunction after revascularization correlates with the proportion of viable myocardial tissue. The most important indication of viability study is to differentiate scarred tissue from living (hibernated) tissue before revascularization.

The gold standard in viability study is the combined evaluation of recent SPECT or PET myocardial perfusion imaging with resting F-18 FDG PET metabolism images. The presence of viable myocardial tissue in patients diagnosed with chronic ischemic heart disease reveals the necessity of revascularization. In addition, the presence of viable tissue indicates that perioperative mortality and morbidity will be lower in these patients and the left ventricular ejection fraction will be higher after revascularization.

Fasting for 6-12 hours is required before F-18 FDG PET imaging for cardiac viability. Basal blood glucose level is measured before the examination. Myocardial tissue uses a higher amount of free fatty acids to provide energy in case of starvation. This rate is around 65-70%. However, under ischemia and hypoxia conditions, the use of glucose as an energy substrate by anaerobic glycolysis increases. To change this situation in favor of glucose, it is necessary to ensure the postprandial state and ensure that the metabolism comes under insulin dominance. Various methods are available to enable myocardial tissue to use glucose. These methods are oral glucose loading, intravenous glucose loading and the use of nicotinic acid derivatives.

When perfusion and metabolism studies are evaluated together, there are three possibilities. The first of these is the study in which both are normal. According to this scenario, the myocardium is viable. The second is the observation of normal or increased metabolic activity in the metabolism study performed with FDG along with decreased blood flow during the perfusion study. According to this scenario, the myocardium is viable. The third is the presence of decreased blood flow in the perfusion study and decreased metabolic activity in the metabolism study with FDG. According to this scenario, the myocardium is not viable.

F-18 Choline PET/CT

Positron Emission Tomography is an imaging method with higher resolution than scintigraphic imaging and can also detect small parathyroid adenomas that cannot be detected in scintigraphy. PET radiopharmaceuticals used in parathyroid imaging are 18F-fluorodeoxyglucose (FDG), 11C-methionine, 11C-choline, and less commonly 18F-DOPA. Choline, the most commonly used PET agent, is a proliferation marker and can be labeled with F18 or C11. It is the precursor of phosphotidylcholine in the cell membrane; It shows increased uptake in hyperfunctioning parathyroid adenomas.

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CHAPTER 7

NON-INVASIVE BRAIN STIMULATION TREATMENT METHODS IN ALZHEIMER'S DISEASE

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Introduction

Non-invasive brain stimulation (NIBS) is a field of neuroscience that explores various techniques to modulate brain activity without surgery or invasive procedures (Medeiros, Barros, & Caixeta, 2023). While NIBS has shown promise in treating a variety of neurological and psychiatric conditions, its application in Alzheimer's disease (AD) is an area of ongoing research. (Hanoglu, Velioglu, Hanoglu, & Yulug, 2023). AD is a progressive neurodegenerative disorder characterized by cognitive decline and memory impairment (Turkseven et al., 2017). NIBS methods aim to slow down or potentially reverse these symptoms by influencing neural activity. NIBS techniques used in AD are mostly preferred as rTMS, tDCS and tACS. From this perspective, NIBS techniques can be categorized into two main groups: transcranial magnetic stimulation (TMS), which uses magnetic fields to modulate brain excitability, and transcranial electrical stimulation (TES), which delivers direct electrical currents to the scalp for neural modulation. TES techniques include transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS) (Medeiros et al., 2023). Here are some NIBS methods that have been studied in the context of AD: Repetitive Transcranial Magnetic Stimulation (rTMS), Transcranial Direct Current Stimulation (tDCS), Transcranial Alternating Current Stimulation (tACS) (Abbasi et al., 2023; Cappon et al., 2023; Hu, Huang, He, & Wu, 2023). rTMS involves using magnetic fields to stimulate specific brain regions (Li et al., 2023). Studies have explored its potential in improving memory and cognitive function in individuals with AD (Hanoglu, Velioglu, Hanoglu, & Yulug, 2023). The results have been mixed, with some studies showing modest benefits (Satorres et al., 2022). tDCS involves applying a low electrical current to the scalp using electrodes. It can be used to modulate neural excitability. Research into tDCS for Alzheimer's has shown varying results, with some studies suggesting it may enhance cognitive function and others showing limited effects (Antonenko & Floel, 2016; Dan, 2017; Weiler, Stieger, Long, & Rapp, 2020; Zhao et al., 2017). tACS uses alternating current to entrain or synchronize brain oscillations in specific frequency ranges. It has been investigated for its potential to improve memory and cognitive function in Alzheimer's patients. Early studies suggest that it might have some positive effects, but more research is needed (Christoph & Daniel, 2017; Lang, Gan, Alrazi, & Monchi, 2019; Stefan et al., 2019). Some research has suggested that TMS may have a neuroprotective effect and could potentially benefit individuals with AD, but more studies are needed to confirm its efficacy. Because AD is a complex disorder. To define it, AD is a chronic neurodegenerative disease and is characterized with the loss of cholinergic neurons, cerebrovascular inflammation and with the inflammation of amyloid beta (Aβ) plaques, ne-

urofibrillary tangle accumulation in cerebral blood vessels and in the brain parenchyma (Turkseven, 2017). The most distinctive neuropathological change in Alzheimer patients is the intense senile amyloid plaques especially in the hippocampus and cortex. Aβ peptide is the major component of senile plaques, and have many toxic effects on neurons, astrocytes, glial cells and brain endothelium. Additionally, recent studies have shed light on oxidative stress and neuroinflammatory pathways (Turkseven, 2017). Therefore, AD is a complex condition with multiple underlying causes, and there is currently no cure. In Alzheimer's Disease (AD), non-modifiable risk factors include age, family history, cardiovascular pathologies, and genetic factors, while modifiable risk factors comprise poor dietary habits, lack of physical exercise, and exposure to environmental stress. From this perspective, non-pharmacological treatments may also play a protective role in overall lifestyle. Therefore, non-invasive brain stimulation (NIBS) has garnered significant interest (Menardi, Dotti, Ambrosini, & Vallesi, 2022). NIBS methods, if proven effective, may offer a complementary approach to existing treatments, such as medication and cognitive therapy, but they are unlikely to provide a complete solution on their own. Also, It's important to note that while NIBS methods are considered non-invasive, they are not without risks and side effects. These side effects can vary from person to person and may differ depending on the applied method, dosage, frequency, and the individual's overall health condition. Here are the potential side effects of NIBS: Headaches: Headaches are a common side effect of NIBS applications. Individuals may experience a sensation of headache when techniques like TMS, tDCS, or other NIBS methods are applied. Skin Irritation: Electrodes used in NIBS methods like tDCS can cause skin irritation or redness. Dizziness and Lightheadedness: Some individuals may experience symptoms like dizziness, lightheadedness, or a loss of balance when NIBS is applied. Involuntary Muscle Contractions: During TMS or tACS applications, there may be involuntary muscle contractions in other muscle groups. Fatigue and Weakness: Some people may feel a short-term sense of fatigue or weakness after NIBS application. Hearing Issues: High-energy TMS applications can lead to temporary hearing issues. Irritability and Emotional Changes: NIBS methods like TMS and tDCS can alter brain activity, potentially leading to emotional changes or irritability in some individuals (Antal et al., 2017; Russo, Souza Carneiro, Bolognini, & Fregni, 2017; Rodriguez, Opisso, Pascual-Leone, & Soler, 2014). The side effects of NIBS methods are generally mild and transient throughout the course of treatment. However, before undergoing NIBS, a person's medical history and current health status should be taken into account. The application should be carefully managed by trained professionals to minimize side effects. If someone is considering NIBS, they should consult with a healthcare professional who is knowledgeable in the

field and be informed about potential risks and benefits. These treatments should only be administered by trained professionals, and their safety and effectiveness in AD are still being studied. Patients with AD should consult with healthcare professionals to explore all available treatment options and participate in clinical trials if they are interested in experimental treatments like NIBS. In this section, non-invasive brain stimulation (NIBS) methods proposed up to the present day for the study of brain diseases, particularly AD pathology, were examined and compared. By doing this, the aim was to highlight the significance of NIBS application in elucidating pathological alterations in dementia and its role in the differential diagnosis and treatment of AD.

Repetitive Transcranial Magnetic Stimulation (rTMS) in Alzheimer's Disease

rTMS is one of the growing non-invasive brain stimulation techniques developed to treat various neurocognitive disorders, including Alzheimer's disease (AD). Although small clinical studies in AD have reported positive effects on cognitive outcome measures, significant knowledge gaps persist, and there has been limited interest in investigating the potential impact of rTMS on AD pathogenesis (Weiler et al., 2020). However, rTMS has shown great potential in the treatment of AD (Zhang et al., 2022). Furthermore, Transcranial Magnetic Stimulation (TMS) is the most established technique for treating brain disorders, with rTMS being an approved treatment for medication-resistant depression (Bhattacharya et al., 2022). In rTMS treatment, rapidly changing electrical current is passed through a coil placed near the skull. This generates a rapidly changing magnetic field, which, in turn, creates an electric field in the cortex, influencing neural activity. Despite significant progress in the use of rTMS in recent years, the underlying mechanisms and optimal operating parameters remain uncertain (Abbasi et al., 2023), as the size of the induced current in the brain can vary (Bhattacharya et al., 2022). It is not well understood which types of neurons are stimulated by TMS and whether the effects of TMS on neurons are excitatory, inhibitory, or context-dependent. It has been reported that TMS primarily affects cortical structures and that targeting specific subcortical regions with TMS is challenging, with TMS pulses unable to stimulate subcortical areas without affecting cortical regions (Bhattacharya et al., 2022). Previous studies have shown that high-frequency (>5 Hz) rTMS increases cortical excitability, while low-frequency (1 Hz or lower) rTMS reduces cortical excitability (Chen et al., 1997; Gangitano et al., 2002; Pascual et al., 1994; Valero-Cabre, Amengual, Stengel, Pascual-Leone, & Coubard, 2017). In light of this information, Zhang et al. conducted a randomized, sham-controlled clinical trial on 35 patients with moderate to severe Alzheimer's disease (AD). Over a three-month period, they ad-

ministered a treatment procedure involving high-frequency (10 Hz) stimulation of the left dorsolateral prefrontal cortex (DLPFC) for 60 sessions. The results of rTMS treatment showed a significant improvement in cognitive performance, a reduction in psychiatric symptoms, and an improved clinician's global impression of change in AD patients (Zhang et al., 2022). Additionally, Wei et al. conducted a meta-analysis, including a total of 513 AD patients from 14 studies. They found that rTMS significantly improved global cognitive function and daily life abilities in AD patients. However, it did not lead to improvements in language, memory, executive ability, and mood (Wei et al., 2022). Further analyses revealed that rTMS at 10 Hz, applied to a single target with 20 treatment sessions, produced a positive effect, and the improvement in cognitive functions lasted for at least 6 weeks (Wei et al., 2022). In their study, Cotelli et al. found that patients with mild to moderate Alzheimer's disease (AD) showed improved action and object naming abilities during the application of 20 Hz rTMS over the dorsolateral prefrontal cortex (Cotelli et al., 2006; Cotelli, Manenti, Cappa, Zanetti, & Miniussi, 2008). Furthermore, Cotelli and colleagues observed long-term effects on auditory sentence comprehension performance in AD patients following the application of 20 Hz rTMS (25 min/day, 5 days/week) (Cotelli et al., 2011). In a study conducted by Ahmed et al., 32 patients with mild to moderate AD and 13 with severe AD received 20 Hz (5s, 20 trains, 5 days) and 1 Hz (2 trains of 1000 s, 30 s intertrain interval, 5 days) rTMS over the dorsolateral prefrontal cortex. They assessed the patients at baseline, 1 month, and 3 months after treatment. The evaluation revealed that high-frequency TMS has long-lasting effects in individuals with mild to moderate AD and is more effective than low-frequency stimulation. It was observed that there was no improvement in severe AD patients (Ahmed, Darwish, Khedr, El Serogy, & Ali, 2012). Notably, The use of rTMS in AD research is still controversial due to protocol differences, such as the standardization of stimulation parameters (intensity, frequency, duration of intervention), determining the optimal stimulation site, and defining a diagnostic framework guided by biomarkers (Menardi et al., 2022). Due to these limitations, the FDA has not yet approved the commercial use of TMS devices for the treatment of AD pathology because the amount of evidence collected so far is not sufficient to clearly indicate its clinical effectiveness (Payesko, 2019). Further research will be necessary to better clarify which protocol features and parameter combinations are most effective in supporting the remaining cognitive functions in AD patients. Most importantly, standardized approaches need to be developed to reduce inter-study heterogeneity and promote reliable findings.

Transcranial Direct Current Stimulation (tDCS) in Alzheimer's Disease

tDCS is a tool that triggers neuroplasticity and modulates cortical function by applying weak direct current to the participants' scalp. It has been widely used in the past decade and has made significant contributions to the fields of neuroscience and psychology (Zhao et al., 2017). tDCS is a commonly employed Non-Invasive Brain Stimulation (NIBS) technique that stimulates the brain by delivering electrical current (1-2 mA) through electrodes (anode and cathode) placed on the scalp (Bhattacharya et al., 2022). Studies have shown that anodal and cathodal electrodes have both depolarizing and hyperpolarizing effects on membrane potential. In a human study, it was found that blocking NMDA receptors with dextromethorphan prevented the plasticity induced by anodal and cathodal tDCS. Additionally, the NMDA receptor agonist D-cycloserine was observed to increase excitability induced by anodal tDCS (22)(Bhattacharya et al., 2022). Activation of NMDA receptors is required for the flow of Ca^{2+} ions into cells, as demonstrated in studies using patch-clamp techniques, for the generation of long-term potentiation (LTP) and long-term depression (LTD) (Bhattacharya et al., 2022). Accordingly, it has been determined that NMDA receptors accompany the synaptic plasticity induced by tDCS. However, cellular studies conducted outside of tDCS applications have indicated that the NMDA receptor is implicated in the excitotoxicity hypothesis, which is among the pathophysiological theories of Alzheimer's disease (AD). Accumulation of amyloid beta, a major hallmark of AD, leads to the formation of free radicals. These free radicals are released into the extracellular space, inhibiting glutamate uptake receptors located on glial cell membranes, which are responsible for reuptake of the neurotransmitter glutamate at synaptic junctions. Unreuptaken glutamate excessively stimulates NMDA receptors, leading to excitotoxicity. With increased excitability, NMDA receptors allow an excessive influx of Ca^{2+} ions into the cell. The excess Ca^{2+} ions are drawn into the mitochondria, especially, causing disruption of mitochondrial structure and initiating apoptosis through the activation of cytochrome c (Turkseven, 2019). In light of these findings, it is possible that the depolarization induced by anodal tDCS may also contribute to excitotoxicity caused by NMDA receptor activation. Nevertheless, research has shown that tDCS applied to Alzheimer's patients produces positive effects in the treatment of elderly adults with memory loss or dementia. Anodal tDCS applied to the temporopolar cortex (TPC) in 10 AD patients was evaluated, and it was found that all patients showed significant improvement in recognition memory performance following active anodal tDCS on the TPC area, suggesting that the effects of tDCS on the TPC are likely specific to recognition memory (Ferrucci et al., 2008a). Si-

milarly, Boggio et al. evaluated 10 AD patients using anodal tDCS on the left temporal cortex and left dorsolateral prefrontal cortex, finding a significant positive effect of tDCS stimulation on short-term memory (visual recognition memory) (Boggio et al., 2009). Additionally, they examined the long-term effect of anodal tDCS on the temporal cortex on visual recognition memory tasks in 15 AD patients after five consecutive sessions. They found that the performance of AD patients in visual recognition memory tasks improved, and this improvement persisted for at least 4 weeks after treatment (Boggio et al., 2012). Khedr et al. also investigated the long-term effectiveness of anodal tDCS on the left dorsolateral prefrontal cortex (2 mA, 25 minutes, and 10 days) in AD neurorehabilitation. The results showed a significant improvement in cognitive performance in AD patients after tDCS application, along with a decrease in P300 latency, an objective biological marker of AD (Khedr et al., 2014). However, the same protocol applied by Cotelli and colleagues, as well as by Suemoto and colleagues, showed negative results (Cotelli et al., 2014; Suemoto et al., 2014). In light of these findings, tDCS is considered a promising NIBS technique for use in AD. Despite the numerous tDCS studies, the underlying mechanisms and neural relationships of tDCS remain not fully understood. Further research should focus on the integration of tDCS with molecular studies to improve cognitive rehabilitation in AD patients in the future.

Transcranial Alternating Current Stimulation (tACS) in Alzheimer's Disease

tACS, a NIBS (Non-Invasive Brain Stimulation) technique, involves the application of oscillating electrical currents to the brain through two electrodes, with the current and polarity changing in accordance with a sinusoidal waveform. tACS can be applied at any frequency (Bhattacharya et al., 2022). It has shown promise as an intervention for individuals suffering from neurodegenerative cognitive disorders. It has demonstrated the potential to enhance cognitive and memory processes in elderly adults experiencing age-related cognitive decline. tACS has been shown to modulate known oscillatory deviations occurring in individuals with mild cognitive impairment and Alzheimer's disease (AD), restoring cortical oscillatory patterns associated with successful cognitive and memory performance. In these patients, evidence of tACS-induced cognitive improvements is particularly associated with gamma stimulation, a frequency range prominently involved in hippocampal-mediated memory processes, often disrupted, especially in the early stages of the disease (Nissim, Pham, Poddar, Blutt, & Hamilton, 2023). Studies in animal models of AD have shown that the restoration of gamma oscillations driven by tACS reduces the pathogenic burden of β-amyloid and significantly improves behavior. Neuroimaging evidence also indicates that the left angular gyrus is a crucial node in the

memory network, and a decrease in gray matter volume in the left angular gyrus is associated with AD memory symptoms. In light of these findings, tACS has attracted interest due to its ability to modulate cortical excitability and safely modulate brain activity at a specific frequency in targeted brain structures, potentially enhancing cognitive functions (Cappon et al., 2023). Recent studies in AD have shown that tACS applied at gamma frequencies targeting key nodes of the memory network can improve episodic memory and restore cholinergic neurotransmission (Benussi et al., 2021; Kim, Kim, Jeong, Roh, & Kim, 2021; Zhou et al., 2022). In a pilot study conducted by Sprugnoli and colleagues, multiple 40 Hz tACS sessions targeting the temporal lobe in mild to moderate AD patients (over 2-4 weeks, 1-hour sessions) were investigated for their effects on cerebral perfusion measured via arterial spin labeling (ASL) MRI, neurophysiology measured via EEG, and episodic memory performance. ASL MRI revealed a significant increase in bilateral temporal lobe perfusion from baseline to post-intervention, and these perfusion changes were positively associated with changes in episodic memory performance and gamma spectral power (Sprugnoli et al., 2021). In another randomized, double-blind, sham-controlled crossover pilot study conducted by Benussi and colleagues, the effects of 40 Hz gamma tACS (at 3.0 mA peak-to-peak intensity) were investigated in 20 AD patients. The active gamma tACS session targeted Pz, a critical node in the episodic memory network, and results demonstrated significant improvements in auditory verbal learning and long-delay recall scores compared to sham stimulation (Benussi et al., 2021). In another study by Benussi and team, 60 AD patients were examined with 40 Hz gamma tACS targeting the precuneus (at 3.0 mA peak-to-peak intensity, lasting 1 hour). The effects of tACS were evaluated on episodic memory and cholinergic transmission, and a significant correlation was observed between the increase in episodic memory and indirect measures of cholinergic neurotransmission following active gamma tACS. Results were supported by changes in EEG indicative of gamma frequency entrainment, and increased gamma activity was observed specifically in the posterior parietal cortex and precuneus, suggesting region-specific stimulation effects (Benussi et al., 2022). These promising results in AD patients indicate that tACS, when used to enhance gamma oscillatory activity in brain regions affected by AD, holds therapeutic potential in the context of memory processing. However, despite these promising findings, tACS, which is increasingly being used to explore and treat neurodegenerative disorders, still retains uncertainty regarding how it affects the dynamics and connectivity of neural circuits.

Conclusions

Alzheimer's disease (AD) represents the most common type of neurodegenerative diseases. Due to its multifactorial nature, it is associated with

the interaction of multiple pathological changes, eventually leading to the clinical diagnosis of dementia, including gradual cognitive decline, brain atrophy, amyloid plaque accumulation, and neurofibrillary tangle formation (Menardi et al., 2022). The National Institute on Aging and Alzheimer's Association (NIA-AA) recognizes AD as a spectrum characterized by widespread cognitive deficits extending beyond well-known memory decline (Jack et al., 2018). The most important findings of the AD are loss of memory, difficulty in performing daily activities, and disruptions in speaking and visual perception. However, very little is known about the causes of Alzheimer's disease, and there are no curative treatments available (Scheltens et al., 2016). Non-pharmacological interventions, along with the effective management of overall health conditions and cognitive health, can play a significant role, particularly in preventive medicine through lifestyle choices. As a form of non-pharmacological intervention, non-invasive brain stimulation techniques (NIBS) have gained significant attention (Menardi et al., 2022). rTMS, tDCS and tACS, which are applications of NIBS, are part of the growing family of non-invasive brain stimulation techniques developed to treat various neurocognitive disorders, including Alzheimer's disease (AD) (Medeiros et al., 2023; Weiler et al., 2020). When these applications are considered in sequence, it has been demonstrated that rTMS can significantly improve cognition in Alzheimer's disease (AD) patients (Cheng et al., 2018). However, it is not clear which brain areas are most suitable for targeting. Structures crucial for memory, such as the hippocampus, are affected in the early stages of pathology (Frisoni, Prestia, Rasser, Bonetti, & Thompson, 2009). Targeting deep brain structures with traditional rTMS coils is challenging, and deep rTMS coils can achieve this with a loss of focus. Therefore, the most commonly targeted cortical region is the dorsolateral prefrontal cortex (DLPFC). However, there is a lack of research on other areas that can provide evidence of effectiveness in the treatment of cognitive decline. Deep rTMS methods have not been reviewed. In individuals with AD, there is a connection between impaired DLPFC plasticity and worsening working memory and language comprehension. Therefore, targeting the DLPFC in this context may be beneficial. Interestingly, rTMS targeting the DLPFC is also an FDA-approved treatment for patients suffering from Major Depressive Disorder. Depression has a high comorbidity with AD, and cognitive impairment is closely associated with depressive symptoms. In an AD study where patients exhibited depressive symptoms, stimulation of the DLPFC improved cognitive performance while also reducing depression. Currently, there is inconsistency among studies. Some exclude patients with depressive symptoms, while others do not specify whether neuropsychological tests are taken into account. These variations make it challenging to investigate the effects of rTMS on cognitive enhancement, as the reduction of depressive

symptoms can contribute to improvements (Heath, Taylor, & McNerney, 2018). The primary mechanism of rTMS is to promote plasticity and strengthen brain connections, and as demonstrated in healthy adults, rTMS can alter cortico-hippocampal connectivity and enhance cognition (Wang et al., 2014). One study on Alzheimer's disease (AD) has reported a disruption in the connectivity between the hippocampus and various cortical regions, including the medial prefrontal cortex, cingulate cortex, inferior temporal cortex, and superior temporal gyrus. Another study has indicated that the decreasing connectivity in the default mode network within cortical structures, including the medial and paracingulate gyri, precuneus, and superior temporal lobe, is associated with progressive decline. Targeting these regions has the potential to directly impact the disrupted connectivity (Heath et al., 2018). In a study by Koch et al., it was found that stimulation of the precuneus region at a frequency of 20 Hz (40 trains, for 2 s, 1600 pulses/d/2 weeks) resulted in improved episodic memory compared to pre-treatment scores in Alzheimer's disease (AD) patients, while no significant difference was observed following sham stimulation (Koch et al., 2018). In another study, involving 34 patients with mild cognitive impairment, after categorizing them into sham and stimulation groups, stimulation of the left dorsolateral prefrontal cortex (DLPFC) at a frequency of 10 Hz (5 seconds, 25 seconds intertrain interval, 20 minutes per day for 5 days per week for 2 weeks) led to significant benefits in daily memory tests compared to the sham group, and these effects persisted for up to one month. However, in this case, scores for logical memory, executive function, and language in the sham group remained stable throughout the observation period (Drumond Marra et al., 2015). In line with this, there is substantial evidence supporting the potential benefits of rTMS for AD patients. But further research is needed to determine which specific brain area is key to effectively improving cognition in Alzheimer's patients (Heath et al., 2018). Another non-invasive technique apart from TMS, known as tDCS, may be advantageous due to its low cost, portability, tolerability, and potential for combination with pharmacotherapy (9)(Zhao et al., 2017). Furthermore, tDCS is considered safer than TMS, which has been associated with the potential to induce seizures when applied inappropriately (56) (Classen et al., 1995). Studies have shown positive effects of tDCS in treating memory loss or dementia in older adults (Elder & Taylor, 2014; Hsu, Ku, Zanto, & Gazzaley, 2015). tDCS has been suggested as an alternative or complementary treatment option for AD patients. Researchers have also attempted to combine tDCS with cognitive training (CT) to achieve sustained and long-term therapeutic effects. In a study conducted by Penolazzi et al., they examined the cognitive effects of two tDCS cycles with CT treatments administered two months apart. They found that tDCS + CT could stabilize global cognitive function for approximately three months,

longer than the effect produced by CT alone (Penolazzi et al., 2015). Therefore, it has been observed that the synergistic use of tDCS and CT can slow cognitive decline in AD patients (Penolazzi et al., 2015). Furthermore, while TMS studies have typically yielded favorable results when targeting the left DLPFC, in research aimed at improving cognitive memory in AD patients, tDCS stimulation of the left TPC/temporal cortex has been found to produce better results compared to the left DLPFC (Zhao et al., 2017). These findings indicate the potential value of tDCS as a tool for cognitive rehabilitation in AD patients. Additionally, transcranial Alternating Current Stimulation (tACS), which generates oscillating electrical currents in the brain, is a subtype of tDCS (Bhattacharya et al., 2022). tACS has recently gained attention in the scientific community due to its ability to entrain gamma oscillations frequently disrupted in AD, low cost, and potential for home-based applications (Dhaynaut et al., 2022). Recent studies in AD have shown that gamma-tACS applied over the precuneus can improve memory performance and restore cholinergic transmission, while gamma-tACS targeting temporal regions can increase blood perfusion and reduce tau burden in these regions (Benussi et al., 2021; Benussi et al., 2022; Dhaynaut et al., 2022; Sprugnoli et al., 2021). In a study, when gamma-tACS was applied, it was observed that the connectivity of active brain regions during resting-state and not engaged in a specific task increased with other large-scale network connections, along with the modulation of inter-network relationships (Altomare et al., 2023). Regarding AD biomarkers, previous studies had observed a reduction in amyloid burden associated with gamma entrainment in mouse models of AD (Iaccarino et al., 2016; Martorell et al., 2019). An earlier pilot study found that gamma-tACS applied to the bilateral temporal lobes in mild to moderate Alzheimer's patients reduced tau burden but did not reduce amyloid burden (Dhaynaut et al., 2022). An increase in cholinergic transmission has also been noted (Benussi et al., 2021; Benussi et al., 2022). In light of this information, due to its specific impact on Alzheimer's pathophysiology, gamma-tACS may have a greater effect on memory rather than other cognitive functions. Such cognitive effects can indirectly improve health-related measures, such as quality of life and independence in daily activities, and reduce the burden on caregivers. Electrical stimulation techniques (tDCS, tACS) have the advantage of lower cost, portability, applicability at home, and can be combined with training or rehabilitation. Affordable home-based treatments based on daily TES sessions may become a reality for the treatment of AD patients and can be applied either as a standalone treatment or in combination with other therapies, such as cognitive stimulation or cognitive-enhancing medications.

The available results present an exciting prospect regarding NIBS as a potentially significant therapeutic approach in the management of Alzheimer's disease. This non-invasive brain stimulation method may pave an innovative path for treating cognitive impairments. Current treatment options for cognitive decline associated with Alzheimer's disease are limited, hence promising strategies like NIBS hold the potential to enhance patients' quality of life and improve their cognitive functions. The growing interest in this research field reflects the efforts to comprehend the intricate nature of Alzheimer's disease and develop effective treatments. However, it should be noted that further data and clinical studies are required in this area. NIBS shows promise as a therapeutic approach for cognitive impairment and mild cognitive impairment in Alzheimer's disease.

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CHAPTER 8

REGULATORY T CELLS' IMPORTANT PARTIES IN IMMUNOTHERAPY AND THEIR THERAPEUTICAL ACTIVITIES IN CANCER

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INTRODUCTION

Therefore, organisms employ the human body as a host to achieve their shared objective of survival. Because of this, the human body is constantly invaded by outside agents, and the body develops an immune system to protect against such threats. Similar to this, the immune system is a mechanism that can identify numerous alien infections and provide various reactions to ward off these pathogens from attacking the organism (Zou W.,2006). The immune system has created a number of effector mechanisms to stop autoimmunity and aid in tissue repair at the same time. The immune system has a number of defense mechanisms that it can activate to stop cancer, inflammatory disorders, and tissue damage and dysfunction. These host defense mechanisms are centered on CD4+ regulatory T cells (Treg) (Kennedy R, Celis E, 2008).

DEVELOPMENT, PROLIFERATION, ACTIVATION OF TREG CELLS

Even though there are few naive T cells that react to a specific peptide antigen, they must grow in quantity in order to kill infections. Therefore, activation by co-stimulatory receptors as well as the combination of TCR peptide and MHC complex are necessary for the clonal proliferation of T cells. The secondary messenger cascade that results from the activation of TCR and CD28 results in the production of IL-2. For T cell proliferation, IL-2 is crucial (Ghiringhelli F, 2007). The high affinity IL-2 receptor has been upregulated as an additional effect. Therefore, a local increase in this cytokine can trigger a response from activated T cells. For signal transduction to be amplified, IL-2 receptor mediation is necessary. Utilizing cytoplasmic percutaneous transhepatic cholangiography (PTK), IL-2 receptors start the second messenger cascade to TCR. Interleukin-2 (IL-2/IL-2) and CD25, which are CD4+ T cell components, are both coexpressed by treg cells (Ghiringhelli F, 2007). These cells play crucial roles in immune homeostasis maintenance and self-tolerance of immunological structure (Whiteside TL, 2015). Among the various immune system components that Treg cells regulate are dendritic cells (DC/dendritic cells), macrophages, T cells, neutrophils, and natural killer cells (NK/natural killer). Therefore, it is well established that preventing both autoimmune and chronic inflammatory illnesses requires maintaining a balanced and functional Treg population. These cells are excellent at stopping autoimmune disorders and suppressing aberrant immune responses to their own antigens (Mougiakakos D, et al., 2010). However, their ability to control the immune response to their own antigens might occasionally have unfavorable effects. Forkhead box protein 3 (FoxP3) is required for the transcription of a subtype of Tregs that also forms in the thymus and expresses CD4 and CD25 on their

surface (Borsellino G, et al., 2007). FoxP3 is the most accurate marker of CD4+ Treg cells in mice and humans, according to studies. FoxP3 in humans and mice differs from one another, albeit (Long SA, Buckner JH, 2011). Two isoforms of the human FoxP3 protein exist, one of which codes for the entire protein and the other of which codes for a shortened version of the protein without exon 2 (Long SA, Buckner JH, 2011). The short version of human FoxP3 is not present in mouse CD4+ Treg cells, though. Human CD4+ T cell FoxP3 expression is dependent on TCR activation, which is another distinction between human and murine FoxP3 (Long SA, Buckner JH, 2011). Treg cell differentiation and operation are controlled by FoxP3. Any human FoxP3 gene mutation has been identified to contribute to X-linked diseases, which are characterized by severe allergies and excessive inflammation. As a result, FoxP3 transcription factor is crucial for the growth of Treg cells. Maintaining peripheral tolerance is done by T-reg cells (Tzankov A, 2008).

The secondary lymphoid organs must include a significant number of mature T cells for proper T cell immunity. These cells have a large number of TCRs that can identify every foreign antigen that might be encountered throughout life. It is crucial that T lymphocytes that recognize foreign antigens spread out to the periphery and remain unresponsive to their own antigens. The most well-known T cell subtypes are those that form in the thymus. The natural killer T cells (NKT) that arise in the thymus have an antigen receptor that has characteristics with both NK and T cells. Thymic progenitor cells produced from bone marrow that reach the thymus through the corticomedullary junction are known as double negative thymocytes because they do not express cytokines like TCR, CD3, CD4, and CD8 during this time. During the first stage of development, they cease to be able to differentiate into other cells and begin to display Thy-1, CD44, and CD25, which are T cell markers. TCR triggers the, loci's TCR gene rearrangement, which spreads from the cortex to the subcapsular zone. The IL-7 receptor mediates communication between notch receptors and particular notch ligands in the control of differentiation and progression in growing T cells. TCR signaling then ensures the growth, survival, and multiplication of T cells (Tzankov A, 2008; Gajewski TF, 2006).

Double negative thymocytes are thymic progenitor cells produced from bone marrow that enter the thymus through the corticomedullary junction and do not express cytokines like TCR, CD3, CD4, and CD8 during this time. In the first stage of their development, they lose the ability to differentiate into other cells and begin to display several T cell markers, including Thy-1, CD44, and CD25. TCR causes TCR gene rearrangement at the, loci, which then travels from the cortex to the subcapsular zone. The IL-7 receptor mediates the signaling between certain notch ligands

and notch receptors in the control of differentiation and progression in developing T cells. Following this step, TCR signaling ensures the growth, proliferating, and surviving of T cells. The development of T lymphocytes requires the passage of two checkpoints. T cells that do not have TCRs that are triggered by the peptide-MHC complex that the thymic ASH presents are first eliminated through apoptosis, and then those that are recognized with low avidity and affinity are changed into single positive mature T cells (positive selection). MHC class I-stimulated T cells become CD8+, whereas MHC class II-stimulated T cells become CD4+. Thymocytes experience apoptosis (negative selection) if they associate with thymic epithelial cells and other thymic ASCs to create a high avidity TCR-peptide MHC complex. Negative selection is a crucial process for the growth of immunological tolerance, despite the fact that it is not always successful. The growth of regulatory T cells (Treg) is the process to remove the effector functions of T cells that exit the thymus in the periphery. The suppression of lymphocyte activity and tumor elimination by a number of Treg cell subgroups has been documented (Long SA, Buckner JH, 2011; Gajewski TF, 2006). The finest examples of this subset are NKT regulatory T cells, naturally occurring CD4+ CD25+ Treg cells, adaptively generated CD4+ Treg cells, and CD8+ Treg cells (Long SA, Buckner JH, 2011). Treg cells can be classified as either adaptive (nTreg) or inducible (iTreg) cells, which represent the two main subgroups of Treg cells. It is unknown exactly how these two types of Treg cells differ from one another. In contrast to iTreg cells, which may tolerate innocuous antigens from some bacterial species that are not their own antigens, nTreg cells are more adept at recognizing their own antigens (Nishikawa H, Sakaguchi S, 2014). However, it is understood that natural killer (NK), natural killer T (NKT), and dendritic cell (DC) function and maturation are inhibited by nTreg cells (Kumai T, et al., 2014). The production of immunoglobulin (Ig), activated B cells, and partially TGF-y secretion are all assumed to be suppressed concurrently by nTreg cells (Kumai T, et al., 2014).

TREG AND CANCER: REGULATORY T CELLS

Even though the effects of Treg cells on cancer are complicated, it has been found that these cells stop the advancement of infiltration into tumour tissues by restricting antitumor immunity and encouraging tumour immune escape (deLeeuw RJ, et al., 2014). Treg cells make up about 4% of CD4+ T cells in the normal state, whereas between 20% to 30% of Treg cells are found in the CD4+ population congregated in tumor microenvironments (Gajewski TF, et al., 2006).

Treg cell populations that are CD4+ and CD25+ predominate in the majority of cancer patients. These cells are quite effective at reducing

immunological responses, according to in vitro tests (Long SA, Buckner JH, 2011). Numerous cancer types' poor prognoses have been linked to the presence of these Treg cells in tumor tissues (Whiteside TL, 2012). Melanoma and non-small cell lung cancer are among these cancer forms. Additionally, it has been discovered that Treg cells in the tumor microenvironment comprise heterogeneous cell subsets that express various immunosuppressive chemicals that aid in the growth of the tumor. In other words, Treg cells are known to create an immunosuppressive milieu that prevents tumor immunotherapy (Mandapathil M, et. al., 2010]. In some cancer types, nevertheless, this situation might be different (Steer HJ, et al., 2010). As an illustration: Additionally, Treg cells have been seen to reduce bacterially generated inflammation in various malignancies, including colorectal carcinoma (CRC/colorectal carcinoma). This encourages carcinogenesis, which is advantageous for the host. An elevated number of Treg cells has been linked to a better prognosis in cancer cases. Treg cells' capacity to reduce overall inflammation leads researchers to draw the conclusion that they promote cell growth and metastasis (Wolf AM, et al., 2003).

THE CAUSE OF THE IMPROVEMENT IN TREG CELL NUCLEATION IN THE TUMOR MICROENVIRONMENT

Chemokines are involved in the infiltration of Treg cells into the tumor microenvironment. For instance, in breast cancer, this happens when CCL22, the ligand of this chemokine released by numerous tumor cells, binds to the chemokine receptor CCR4 secreted by Treg cells (Tan MC, et al., 2009; Yoshie O, Matsushima K, 2015). A cytokine called TGF-/transforming growth factor beta, which is generated by tumor cells, promotes Treg cell development and activation. Additionally, it is thought that the production of Treg cells from CD4+ CD25+ T cells in the periphery may be the cause of the high levels of Treg cells that have accumulated in the tumour microenvironment (Camisaschi C, et al., 2010).

Both to offer antigenic stimulation for T cell activation and to release cytokines crucial for T cell development, tumor cells interact with innate immune cells that have infiltrated the tumor. Type 1 helper T cells (Th1/T helper1), type 2 helper T cells (Th2/T helper2), Treg cells, and interleukin-17 (IL-17/interleukin-17) generating T cells (Th17/T helper17) can all be produced from naive CD4+ T cells. The degree of antigen stimulation and the cytokine environment influence this differentiation. TGF- stimulates the conversion of naive T cells into Treg cells and suppresses the development of Th1 and Th2 cells when combined with poor antigen stimulation. Th17 hucrelerine dönüşümünü desteklemektedir. TGF- ile interlökin-6 (IL-6/interleukin-6). In addition, dorudan timüs tarafndan üretilmiş

olan CD4+ CD25+ Treg hücreleri, tümör mikro çevresinde birikmektedir (Dahmani A, Delisle J-S, 2018; Wang R, et al., 2008). Kanser hücreleri tarafndan eksprese edilen baz antijenler ile çapraz reaksiyona girebilen.

Targeting TREG and using immunotherapy

Weakened immunity is one of the characteristics of cancerous cells. The body creates defense mechanisms to stop this and eliminate tumor cells. Treg cells are crucial in preventing effector cells in the tumor microenvironment from functioning to their full potential. Continued growth in the field of study on the modulation of Treg cell suppression or decrease in the microenvironment of tumors (Vesely MD, et al., 2011). Studies have demonstrated that systematically decreasing Treg cells boosted the anti-tumor response (Zou W, 2006; Colombo MP, Piconese S, 2007; Pickup MW, 2017). Autoimmunity, however, appears when Treg cells are reduced systemically. Immune system cells are consequently triggered to produce an immune response against their own antigens after identifying them as alien. Because of this, the body starts to attack its own antigens, leading to the development of autoimmune illnesses. By using CD25 monoclonal antibody (mAb), Treg cells were targeted in order to overcome immunological insensitivity to tumor cells (Schmidt A, et al., 2012). Natural killer cells (NK) and CD8+ effector T cells that are specific for the tumor are also stimulated to develop on their own. Depletion of Treg cells, however, has produced cross-reactive immunity against many tumors. Additionally, tumor regression was not seen when CD25 mAb was given without Treg cell suppression. On the other hand, after the application of this therapy, the number of Treg cells rose over time and their ability to trigger an anti-tumor response steadily reduced (Valzasina B, et al., 2006; Tan MCB, et al., 2009).

The depletion of effector Treg cells by monoclonal antibodies may be caused by chemokine receptors like CCR4 and several cell surface chemicals produced by effector Treg cells (Kavanagh B, et al., 2008). Use of antagonistic antibodies in immunotherapy is used to disrupt critical immune regulatory molecules (checkpoint molecules), such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) (Pai CCS, et al., 2019). Human CTLA-4 antibodies are known to offer melanoma protection for a lengthy period of time. By preventing the negative signal from B7 CTLA-4 contacts, these antibodies are expected to promote the activation of dormant T cells, resulting in tumor rejection (Schwartz JCD, et al., 2001). Anti-CTLA-4 antibodies are said to suppress Treg cells in the tumor microenvironment in a specific manner. This leads to the conclusion that the tumor is rejected by anti-CTLA-4 antibodies. It is an unconfirmed notion, according to the checkpoint blockade hypothesis, that anti-CTLA-4 anti-

bodies disrupt B7-CTLA-4 signaling in peripheral lymphoid organs and result in tumor rejection by encouraging naive T cell activation (Stamper CC, et al., 2001).

Studies on this topic led researchers to the conclusion that the immunotherapeutic activity of anti-CTLA-4 antibodies does not require blocking the B7-CTLA-4 connection. According to research, it is incorrect to assume that anti-CTLA-4 antibodies cause tumor rejection by encouraging T cell activation in lymphoid organs (Yu C, et al., 2011). Recent research has shown that by eating mice anti-CTLA-4 antibodies, Treg cells in the tumor microenvironment cause tumor rejection. The similarity of the outcomes obtained by mice anti-CTLA-4 antibodies and human anti-CTLA-4 antibodies (Prabhakaran K, et al., 2020) is encouraging.

Due to their immunosuppressive characteristics, treg cells, which are prevalent in the tumor microenvironment, provide the biggest challenge to the efficacy of cancer immunotherapy by promoting tumour resistance in the area. The production of checkpoint molecules such CTLA4, PD1, and LAG3 by Treg cells is the primary factor in their success in cancer immunotherapy (Weinmann SC, Pisetsky DS, 2019). Because of this, a significant rise of detrimental occurrences like autoimmune-related adverse events (IRAE/immune-related adverse events) could result from the widespread use of immunotherapy for the treatment of cancer. Due to this issue, strategies for preserving immune system homeostasis following immunotherapy have been researched (Ondondo B, et al., 2013; Togashi Y, et al., 2019). Understanding the function of Treg cells in sustaining immune system homeostasis and initiating the anti-tumor action is crucial (Spranger S, Gajewski TF, 2018).

To solve this problem, cancer immunotherapy should focus on how to eliminate the suppressive functions of Treg cells (Ward NC, et al., 2020). For this, firstly, CD25+ Treg cells should be eliminated. For this purpose, Treg cells should be exposed to either a specific antibody or IL-2-toxin fusion proteins. However, it is possible to encounter the problem that this approach does not effectively reduce Treg cells or, on the contrary, that Treg cells and activated effector cells are completely eliminated. This may be due to the non-specificity of the CD25 marker for Treg cells. Because CD25 marker is positive in all activated T cells (Rech AJ, Vonderheide RH, 2009). Therefore, a more specific method and research on this subject is needed.

Cancer treatment should concentrate on eradicating Treg cells' suppressive properties to address this issue (Ward NC, et al., 2018). To do this, CD25+ Treg cells must first be removed. Treg cells should be exposed to either a particular antibody or IL-2-toxin fusion proteins for this purpose.

However, it is conceivable to run into the issue where this method either fails to successfully diminish Treg cells or, on the contrary, entirely eradicates Treg cells and activated effector cells. This might be as a result of the CD25 marker for Treg cells' lack of specificity. Because all activated T cells are positive for the CD25 marker (Rech AJ, Vonderheide RH, 2009; Ward NC, et al., 2018). Consequently, a more focused approach and research on this area are required.

CONCLUSION

According to the findings of the investigations, Treg cells in the tumor microenvironment are likely to suppress anti-tumor immunity. The creation of a successful treatment strategy is significantly hampered by this restriction. In addition to attracting Treg cells to the tumor site, tumor cells also facilitate the conversion of dormant or effector T cells into Treg cells using a variety of cytokines and innate immune system cells. Particularly in various cancer types, it has been determined that a low prognosis is associated with a decline in CD8+ T cells when compared to Treg cells.

Understanding the function of Treg cell biology in tumor formation and progression is essential for successful cancer immunotherapy. The most crucial stage in this process is to locate particular Treg cell surface markers. More research is being done on the methods by which Treg cells initiate tumor-specific tolerance. A thorough understanding of Treg cells will result from the creation of novel tactics that reduce the number of Treg cells and interfere with their genesis cascade or function. The negative autoimmune-related effects of targeting Treg cells are one of the most significant challenges for cancer immunotherapy. A difficult part of cancer treatment is this.

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CHAPTER 9

CURRENT APPROACHES TO BONE MINERAL DENSITOMETRY

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Introduction

Bone is an organ with various functions, such as protecting vital organs from trauma, provides mechanical support for soft tissues and is a calcium reservoir, facilitating movement by providing connections to muscles that enable them to move, and supporting hematopoiesis. The human skeleton consists of cortical (80%) and trabecular (20%) bone types. The exterior compact sortical side consists of cortical bone, and the inner region of the bone is supported via trabeculae, called trabecular bone which has a spongy building that forms a web-like shape. Cortical bone forms the tough outer cortex that gives bones a smooth white appearance.

Bone tissue is a modified form of connective tissue with many mechanical and physiological functions, surrounded by a hard and calcified framework consisting of extracellular fluid, cells and fibers. Bone is a living tissue consisting of two main components: an organic collagen matrix and mostly crystallized calcium phosphate. The majority of the bone matrix consists of collagen fibers and non-collagenous proteins. The cells that makeup bone tissue can be listed as osteoblasts, osteoclasts, osteocytes and osteoprogenitor cells.

The development of bone structure is affected genetic structure, acquired diseases, previous operations, number of pregnancies and menopausal status. In addition, people becoming increasingly immobile, decreasing exposure to direct sunlight, long-term medication use, and some chronic diseases affect bone development. To maintain homeostasis, bone tissue constantly renews itself. Following bone resorption by osteoclasts, osteoblasts synthesize collagen matrix at specific sites. In young adults, bone formation nearly equals bone destruction. As we age, bone destruction gradually becomes greater than bone formation and total bone mass begins to decrease. The risk of bone fractures increases with decreasing in bone density.

Osteoporosis is a metabolic progressive systemic bone disease that results in increased fracture tendency and bone fragility in consequence of deterioration of the microarchitecture of bone tissue and low bone mass. Osteoporotic fracture locations in osteoporosis patients include the femur, spine, proximal humerus and distal forearm. These fragility fractures appear as a very important cause of morbidity in our world where average life expectancy and osteoporosis are increasing.

Worldwide, osteoporosis causes 8.9 million fractures annually. Osteoporosis affects nearly 21.2% of women with >50 years and 6.3% of men with >50 years worldwide by using the WHO definition of osteoporosis. According to this calculation, it is a disease that affects approximately 500 million people.

During adolescence and childhood bone density increases until peak bone mass is reached. In adulthood subsequent bone loss and peak bone mass are also major determinants of osteoporosis. Therefore, achieving an optimal peak bone mass in the beginning of life is crucial to minimizing the risk of fractures later in life. Bone formation occurs during the first twenty years of life when bone increases in both size and mass. Bone mineralization begins to decline around age 20 and reaches a plateau at around age 30. This is the point where the skeleton has the highest bone mass and is called "peak bone mass" (PBM). Later on reaching peak bone mass, bone mass starts to weaken at similar stages for both genders.

Osteoporosis is caused by alters in bone remodeling in consequence of the asymetry between bone resorption and formation and the superiortiy of bone resorption leads to a decrease in strength of bone and the fracture occurrence. Bone remodeling is a physiological process whose function is the constant renewal of the skeleton to guarantee biomechanically right bone function. Both resorption and formation are regulated by local and endocrine factors. Vitamin D, estrogen, parathyroid hormone, and to a lesser extent androgens are main endocrine factors. Leptin, growth hormone and thyroid hormones play a lesser role. Various cytokines such as growth factors, TNF-α, IL-1 and IL-6 regulate this process as a local factors.

The final pathway and main regulator of bone remodeling is the NF-κB (receptor activator nuclear factor kappa B) ligand (RANKL) and interaction with it's receptor. RANKL interacts with the receptor called RANK, which is found in both osteoclast precursors and osteoclasts. RANKL is a member of the TNF superfamily of ligands and receptors. Osteocytes are the source of RANKL, which is necessary for osteoclast development during bone remodeling. RANKL also binds to a protein called osteoprotegerin (OPG). Osteoprotegerin inhibits osteoclast activation. RANKL causes osteoclasts activation and differentiation.

Osteoporosis is a bone disease that is very widespread in both women and men today. As life expectancy increases, the frequency of osteoporosis and the number of osteoporosis-related fractures gradually increase. Osteoporosis is a disease characterized by deterioration in the microarchitecture of bone tissue and low bone mass, resulting an increase the risk of fracture and bone fragility.

Osteoporosis is divided into primary and secondary osteoporosis. While primary osteoporosis, the most common type of osteoporosis, is used to describe osteoporosis that develops during normal physiological periods of life, such as menopause and aging, the term secondary osteoporosis is used for osteoporosis that develops not spontaneously but due to a different condition or factor. These different reasons may include var-

ious diseases, long-term medication use, and long-term immobilization. Primary osteoporosis is divided into two: Type I Osteoporosis and Type II Osteoporosis. While Type I Osteoporosis refers to bone loss due to postmenopausal endogenous estrogen deficiency, Type II Osteoporosis refers to age-related osteoporosis that can occur in both genders.

Before the invention of bone mineral density measurement with DXA, the diagnosis of osteoporosis was made by the presence of fragility fractures. The diagnosis of osteoporosis is made by measuring bone mineral density or detecting the presence of a fracture caused by low trauma. Measuring bone mineral density can be done with a bone mineral densitometry device. The radiation dose exposed to this method is quite low. The equivalent dose received by the patient during BMD measurement with DXA is very low, 1-10 μSv, and is at the level of the daily natural radiation dose (7 μSv). At least 20-30% bone mass loss is required for osteoporosis-related changes in the hips or vertebrae to be directly reflected and detected on radiographs.

Bone Mineral Densitometry Measurement

Measurements made with bone mineral densitometer devices are based on the interpretation of the amount of mineral that causes a certain amount of radiation to be absorbed by the bone. Bone mineral densitometry devices work on the principle that when X-rays, gamma rays or sound waves pass through the bone, some of them are absorbed by the bone and the remaining amount of radiation is measured precisely. With the calculations made as a result of this measurement, the mineral content of the bone that causes absorption in unit area or volume is estimated.

Gamma photon absorptiometry, X-ray absorptiometry, quantitative CT or quantitative US methods can be used for bone mineral densitometry measurement. Among these methods, the World Health Organization (WHO) and the International Osteoporosis Foundation (IOF) recommend that the Dual-energy X-ray absorptiometry (DXA) method be chosen as the reference technology in the diagnosis of osteoporosis.

With the DXA method, bone mineral density in various anatomical regions can be measured trabecularly and cortically. Frequently used areas for measurement; lumbar vertebra (L1-L4) and hip (femoral neck and trochanter). Measured values are given as BMC (Bone Mineral Content) in gr or BMD (Bone Mineral Density) in gr/cm^2. BMC is a measure of the amount of minerals found in bone. BMD is the ratio of mineral content measured in a defined area in the bone. In other words, BMD is obtained by dividing BMC by the measured bone area.

DXA Device

A DXA device consists of an X-ray tube, patient examination table, detector, photomultiplier tube system, amplifiers and computer units. Between the X-ray tube and the detector is the examination table on which the patient lies. In DXA devices, the scanning system is in the form of a C arm containing the X-ray tube and detector. The X-ray tube used as the radiation source is located under the C-arm system. In this system, high and low energy rays come out of the X-ray tube at different times.

Detectors that detect X-rays passing through the patient are located in the upper part of the C-arm of the device. The diameter of the detectors is 3 mm and their number is between 40-60 pieces. Sodium iodide (NaI) or a scintillation crystal with similar properties is used in detectors. X-rays detected by the detector hit the scintillation crystal and turn into visible light photons. After the X-rays passing through the patient are converted into light photons in the scintillation crystal, they are converted into electrical signals by photomultiplier tubes and their energy is increased. Electrical signals coming from photomultiplier tubes are amplified in amplifier elements, formatted with other intermediate elements and transferred to the computer unit. The computer unit creates the image by processing the original data transferred to it. The image created on the computer is transferred to the imaging unit.

In most DXA devices, daily calibration is done with a phantom that reflects the representative bone structure provided by the manufacturer. When the device is first delivered or on the day it is maintained and confirmed by the company to have maximum performance, 20 phantom scans should be performed to create a baseline value. The average BMD value of these 20 scans is calculated. By repeating the phantom scan every day with the same phantom and the same scanning speed, it should be checked whether the daily phantom scan value is within ± 1.5% of the average of 20 scans.

The X-ray tube produces X-rays at two different energy levels for soft tissue and cortical bone. Bone and soft tissue separation is achieved by utilizing the attenuation difference that occurs when these two different X-rays pass through various tissues within the body. In this way, bone mineral density is calculated. With the DXA device, first bone mineral content (g) is calculated, and then bone area (cm^2) is calculated. Bone mineral density (gr/cm^2) is calculated by dividing the bone mineral content value by the bone area value.

While imaging is performed on the bone mineral densitometry device, Velcro straps are used to position and fix the patient in the desired position. When performing lumbar vertebra measurements, a special support cush-

ion should be placed under the knees to eliminate lumbar lordosis.

DXA Patient Preparation

- Before the Bone Mineral Densitometer measurement, the patient can continue his normal daily diet on the day of measurement.
- In cases where imaging methods such as barium radiography or tomography or magnetic resonance by injecting contrast material are used, a waiting period of at least 7 days is required before DXA.
- The patient should take care to choose clothes in which he can move comfortably. In addition, one should be careful not to use accessories such as metal jewelry, belts and buttons.
- The patient's weight and height should be measured before imaging.
- The patient's age should be entered into the computer software by looking at the date of birth on his/her ID card.
- It is recommended that at least 7 days elapse after a recent radiological gastrointestinal contrast study and scintigraphic examinations performed with long-lived radioisotopes (such as gallium 67 and iodine 131).
- Tablets containing calcium should not be used for 24 hours before shooting.
- It is recommended that the patient wear comfortable clothing that does not contain metal.

DXA Measurement Areas

During the procedure, the patient is in a supine position. For lumbar vertebra measuring, the patient flexes his hips and knees and a supportive pillow is placed under the patient's legs.

For lumbar vertebrae, measurement should include vertebrae L1-L4 whenever possible. Diagnostic classification should not be made with the BMD value obtained from a single vertebra. In this case, a different skeletal region should be used for diagnosis. It can be used if the t-score is obtained from at least two vertebrae. Vertebral BMD values obtained in the lateral position should not be used for diagnosis but can be used in follow-up. If there is a difference of more than 1.0 between the T scores of two adjacent vertebrae, it should be questioned.

The legs should be in internal rotation when measuring for the femur. In this way, the femoral neck is visualized better in PA images. The foot should be fixed with special straps to prevent movement of the femur. The

left or right femur can be used for BMD measurement. When measuring the femur, the lower total proximal femur or femoral neck measurements should be used.

If hip and vertebra scanning cannot be performed or cannot be interpreted, in the presence of hyperparathyroidism, in severely obese patients exceeding the carrying limits of the DXA device, and in cirrhotic patients with ascites, measurements can be made from the forearm. In this method, the patient is positioned by sitting on a chair and pronating the forearm. To prevent the patient's movement, the forearm is fixed by placing it on a special apparatus called an "arm board". The right and/or left forearm can be used for BMD measurement. However, it is recommended to use the non-dominant forearm. Mid-distal radius and ulna are used for evaluation. Validated 1/3 distal radius measurements should be used for osteoporosis diagnosis and fracture risk assessment. To evaluate the risk of fracture, measurements made from at least two regions must be used.

According to WHO, it is recommended to use the t-score obtained from the femoral neck in the diagnosis of osteoporosis, while according to BHOF and the International Society for Clinical Densitometry (ISCD), it is recommended to use the lowest of the t-scores obtained from the L1-4 vertebrae, total femur proximal and femoral neck. Ward's area and trochanteric area are not recommended for use in the diagnosis of osteoporosis.

t ve z Score:

The t-score is a value with standard deviation as a criterion that compares the patient's BMD value with the young adult's BMD value. It is calculated by subtracting the young normal BMD mean from the patient's BMD value and dividing the resulting value by the standard deviation of the young normal population. If the T score is 0, it means that it has an average value; if it is negative, it means that it is lower than the average value; if it is positive, it means that it has a higher value than the average.

The z-score is the value whose unit is the standard deviation, which indicates the criterion that compares the patient's BMD value with the BMD value of his or her age group. It is calculated by subtracting the age, ethnicity, and sex-matched BMD average of the patient's age group from the patient's BMD value and dividing the resulting value by the standard deviation of the population in his or her age group.

Since various DXA devices use different technologies and databases, it is recommended that comparisons be made on the same device and by the same technician, if possible.

DXA Indications:

According to The National Osteoporosis Foundation, DXA indications are listed as follows:

- Men age ≥ 70 years
- Women age ≥65 years
- Postmenopausal women <65 years, women in the menopausal transition, and men aged 50 to 69 years with clinical risk factors for fracture.
  - Adults who have a fracture after the age of 50 years
  - Adults with a disease or taking a drug (e.g., daily dose of ≥5 mg prednisone glucocorticoids for ≥3 months) associated with bone loss or low bone mass.
  - Low body weight
- Adults with a fragility fracture
- Patient who is about to start medication that could lead to a possible decrease in bone mineral density
- To evaluate the treatment response in patients who started treatment after bone mineral density measurement.
- Anyone not receiving therapy in whom evidence of bone loss would lead to treatment

Long-term use of anticonvulsants, aromatase inhibitor therapy, androgen deprivation therapy, and heparin therapy may negatively affect bone mineral density. Similarly, bone mineral density may be negatively affected in Cushing's syndrome patients, hyperthyroidism and hyperparathyroidism. In cases of long-term immobilization, gastric bypass surgery, malabsorption, malnutrition, organ transplantation, eating disorders, and chronic renal failure may also be affect bone mineral density.

DXA Contraindications

There is no absolute contraindication for DXA imaging. However, DXA should not be performed on pregnant patients if possible because it contains ionizing radiation, even though the radiation dose is very low. If possible, measurement should be postponed until after pregnancy.

DXA Measurement Frequency:

It should be repeated every 2 years for men >70 years and postmenopausal women who are not receiving treatment, once a year for patients under treatment, every 6 months for those receiving "Teriparatide" treatment, and every 6 months or a year for those with secondary osteoporosis and those using glucocorticoids. For follow-up BMD measurements in children and adolescents, the follow-up interval should be at least 6 months.

Since changes in reference databases may cause t-score differences in bone densitometry imaging of patients performed at consecutive/different times, comparison should be made using BMD in g/cm2 instead of t-score. A change in BMD of 3% or less between two measurements is considered a normal variation.

Interpretation of DXA Results:

Diagnosis of osteoporosis is based on bone mineral density measurement or by developing a low-trauma fracture. When interpreting DXA for osteoporosis, t and z scores are used, not BMD. In postmenopausal women and men >50 years the t-score should be used to diagnose osteoporosis. In premenopausal women and men under <50 years z-score should be used to diagnose osteoporosis. T-score should not be used for people who have not yet reached peak bone bass. In these patients, the z-score should be used. The lowest value obtained in each patient should be taken into account.

t-score of -1 and above are classified as "Normal". Patients with a t-score between -1 and -2.5 are classified as "Osteopenia". Patients with a t-score – 2.5 or lower are classified as having “Osteoporosis”. Patients with a t-score of 2.5 or lower and one or more fractures are classified as having “Severe/Established Osteoporosis”. It is preferred to use the terms "low bone mass" or "low bone density" instead of the term osteopenia. In men under the age of 50, osteoporosis cannot be diagnosed based solely on bone mineral density measurement.

If the z-score is -2 SD or below, it is classified as "lower than expected bone mass for chronological age", and if it is above -2, it is classified as "normal bone mass for chronological age". Patients with a Z score >0 are classified as having “higher than normal bone mass for chronological age”.

Patient positioning is of great importance for DXA measurement. Errors related to patient positioning can cause serious reporting errors. For lumbar vertebra measurements, a straight position of the spine should be ensured by flexing the knees to 90 degrees. For hip area measurements, the femur should be internally rotated approximately 15 to 25 degrees. Thanks to this internal rotation, the X-ray will arrive at the femoral neck at a right angle. Whether the femur is sufficiently internally rotated can be determined by looking at the lesser throchanter in the images. If adequate internal rotation is achieved, the lesser trochanter is almost invisible. However, since patient movement during shooting may lead to erroneous evaluations, the patient must remain motionless.

In obese people and in obese people who have lost weight rapidly, the presence of abdominal pannus may lead to erroneous results in images taken from the hip area. Pannus tissue should be removed from the mea-

surement area as much as possible. For BMD measurement, regions of interest (ROI) are drawn in the femur, vertebra and forearm regions and measurements are made from these areas. Drawing these ROIs incorrectly can cause serious errors in BMD measurements. For example, placing the ROI on the T12 vertebra instead of L1 during spinal segmentation will affect the entire measurement.

Imaging with recent radioisotopes or using iodinated contrast agents before DXA imaging may affect BMD measurements. However, recent imaging with gadolinium-containing contrast agents does not have a significant effect on BMD measurement. It has been reported in various studies that oral contrast agents containing barium sulfate may also cause artifactually high BMD measurements. Likewise, the presence of abdominal ascites has a significant impact on lumbar vertebra BMD measurements.

In follow-up patients, the presence of significant weight loss between two measurements may negatively affect the comparison. During lumbar vertebra measurements, any history of fracture, serious degenerative change, Paget's disease, metastasis, laminectomy, spinal fusion or vertebroplasty in any vertebra requires excluding that vertebra from evaluation. Cholelithiasis, nephrolithiasis and calcified intra-abdominal lymph nodes may cause errors in BMD calculation. When evaluating the hip region, the presence of heterotopic ossification may affect the evaluation.

Since metal objects on the patient may negatively affect the evaluation, it is of great importance to remove these objects from the body before measurement. Again, since abdominal aortic calcification may affect lumbar vertebra BMD measurements, it can be easily distinguished with lateral images taken in suspicious cases. Posterior vertebral elements are not present congenitally in spina bifida patients and may cause erroneously low BMD results in BMD measurements.

An issue that should be considered in follow-up BMD measurements is whether there is a difference in patient positioning and drawing areas of interest between the two measurements. Differences in these stages can cause serious evaluation errors. As mentioned before, BMD is obtained by dividing BMC by the area of the drawn region. Therefore, although BMC may be similar between the two measurements, differences in the areas of interest drawing may cause different results in BMD measurements between the two measurements. For this reason, it is of great importance that the area of interest drawings between the two measurements are similar.

FRAX Risk Scoring

Osteoporotic fractures may also be called low-trauma fractures or fragility fractures. These types of fractures occur without major trauma.

These fractures can cause mortality and morbidity in patients. Low bone mineral density increases the risk of fracture.

In 2008, the World Health Organization working group recommended the use of the fracture risk assessment tool (FRAX), which predicts the 10-year hip fracture risk or major osteoporotic fracture risk in patients who have not yet received treatment, using clinical risk factors in people between the ages of 40 and 90. According to FRAX scoring, starting treatment is considered cost-effective if the 10-year risk of hip fracture is ≥3% and the risk of major osteoporotic fracture is ≥20%. In this method, while the femoral neck bone mineral density obtained by DXA is included in the calculation, the values obtained from the lumbar vertebrae are not used.

FRAX calculates the risk using the variables femoral neck bone mineral density, age, gender, body mass index, history of osteoporotic fracture, family history of hip fracture, smoking, glucocorticoid use, presence of rheumatoid arthritis, alcohol use, and presence of secondary causes of osteoporosis. FRAX has been validated in approximately 26 independent cohorts, mainly comprised of women.

Limitations of this scoring include the lack of extensive validation in patients receiving treatment and the lack of validation in the use of BMDs measured with technologies other than DXA. The fact that FRAX is based only on femoral neck measurement with DXA prevents the use of values taken from other areas in this scoring.

In patients with lumbar vertebra BMD lower than femur measurements, in patients with diabetes mellitus, in patients with high-dose glucocorticoid exposure (prednisolone>7,5 mg/day or equivalent), in patients with multiple fractures, in patients with recent fractures, in patients with prevalent, severe vertebral fractures and parental history of non-hip fragility may underestimate the risk of fracture.

Pediatric Osteoporosis

Osteoporosis has started to be encountered in childhood in recent years. The reason for this is that the treatment of chronic diseases encountered in childhood has become complicated and as a result, life expectancy is prolonged and there is sufficient time for the development of osteoporosis. Fractures that occur as a result of osteoporosis can cause pain and limitations in the quality of life in pediatric patients. The most common cause of primary osteoporosis in childhood is Osteogenesis Imperfecta. In this disease, there is an abnormality, especially in Type 1 collagen synthesis and is characterized by decreased bone formation in bone histology.

Osteoporosis-pseudoglioma syndrome is a rare condition characterized by severe thinning of the bones (osteoporosis) and eye abnormalities leading to vision loss. People with this condition are usually diagnosed with osteoporosis in early childhood. It is caused by a decreased bone mineral density. Affected individuals often have multiple bone fractures, and it can cause collapse of the affected vertebrae, scoliosis, short stature, and limb deformities. Decreased bone mineral density can also cause craniotabes.

Many factors lead to childhood osteoporosis, and the risk of osteoporotic fracture development varies with the presence of one or more of these factors. These factors can be listed as decreased mobility, inflammatory cytokines, systemic corticosteroids, problems in puberty, malnutrition or low body weight. Decreased mobility occurs in many diseases encountered in childhood. These are cerebral palsy, spinal cord injury, head trauma, spinal muscular atrophy and various neurodisabilities of unknown cause. As mobility decreases, the load on the lower extremities also decreases, and as the child grows, the weight-bearing long bones turn into a long, thin and flimsy structure. In such patients, fractures occur in the presence of minimal trauma, usually in the distal femur or proximal tibia.

Inflammatory diseases that cause osteoporotic fractures include idiopathic juvenile arthritis, systemic lupus erythematosus and Crohn's disease. In these diseases, increased levels of IL-1, IL-6, IL-7, TNF alpha and TNF beta in the blood disrupt osteoblast functions and stimulate osteoclast production. As a result, bone metabolism is disrupted.

Childhood osteoporosis diagnosis is mainly depending on the presence of fragility fractures. But DXA is suggested to provide an exact evaluation of bone status. In spite of some limitations, DXA is the elected modality for deciding bone health in pediatric population. The total-body-less head and lumbar spine are the favored skeletal areas for measurment because they are the most accurate and consistent areas in pediatric population. Moreover, the z-score should be corrected according to the height of children whose height below the 3rd percentile.

The fact that DXA has high sensitivity and accuracy, that patients are exposed to low radiation doses, and that the procedure is completed in a short time provide important advantages for its use in childhood. However, DXA causes some problems in the diagnosis of childhood osteoporosis due to some of its features. Since the T score is specified for adults, threshold values are not suitable for children.

Additionally, in measurements made with DXA, the results are measured as gm/cm^2 and not gm/cm^3. There is a strong correlation between height and weight in area-based measurements. Short children have small-

er bones, resulting in a shorter child's bone measurement being lower than a taller child of the same age. In this case, short children may be incorrectly diagnosed with osteoporosis and start treatment.

In addition, since the results may be different with different methods, it may be possible that the measurement results do not match between devices of different brands. Therefore, each patient must be measured from the same device. Reference values for the pediatric age group are available for the lumbar vertebrae and the entire skeleton, except the hip. To correctly interpret the data obtained by the DEXA method in children, the length of the measured bone, as well as the patient's height, pubertal stage, skeletal maturation, race and body composition, must be taken into account. Bone density measured in children is evaluated with the Z score.

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