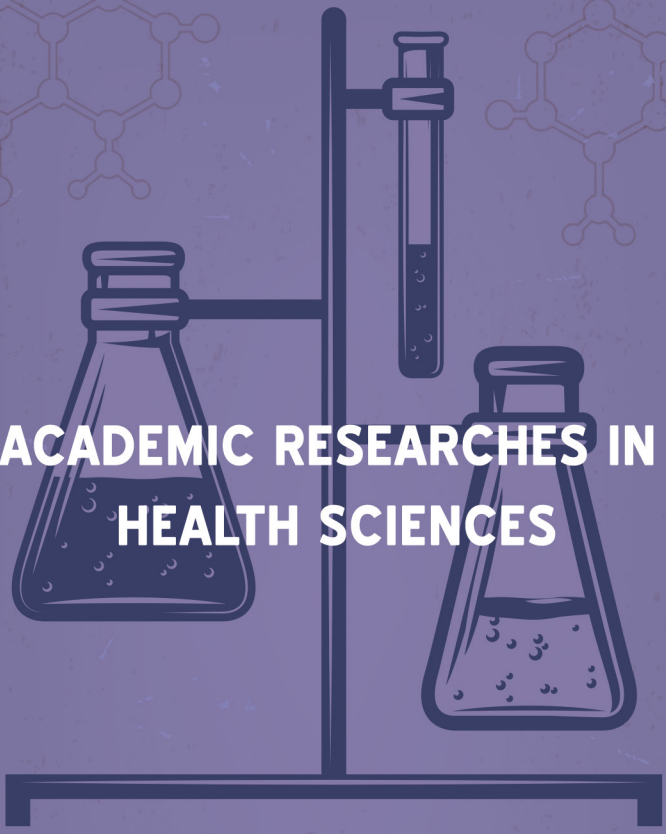
ACADEMIC RESEARCHES IN HEALTH SCIENCES



Editor

Assist Prof. Dilek Atik Ph.D.

DUVAR

ACADEMIC RESEARCHES IN HEALTH SCIENCES

Editor

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CONTENTS

Chapter-1

CURRENT APPROACH TO THE TREATMENT OF NON-ALCOHOLIC FATTY LIVER DİSEASE AND PHARMACOLOGICAL AGENTS UNDER INVESTIGATION

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Non-alcoholic fatty liver disease is the most common chronic liver disease in developed countries and its prevalence in the general population is estimated to be 20-30% [1]. It is a clinicopathological condition in which hepatic fat content generally increases secondary to insulin resistance, except for reasons other than those related to alcohol consumption or other factors causing and secondary liver fattening. NAFLD is characterized by different entities, such as simple steatosis (SS), non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular cancer (HCC). SS is the most common type and has been considered as the most benign presentation of the disease [2]. However, approximately one-third of patients with SS progress to NASH, defined as the presence of NAFL plus inflammation with hepatocyte injury, fibrosis, and cirrhosis or HCC [3,4].

NAFLD is not only the most common chronic liver disease but it also forms the hepatic component of metabolic syndrome and is closely related to the other clinical features of this syndrome [5]. The prevalence of NAFLD is reported to be over 75% in populations in which obesity, metabolic syndrome, hyperlipidemia, and type 2 diabetes mellitus are common [6]. NAFLD has an important clinical presentation because it is associated with insulin resistance, obesity, and metabolic syndrome, increases the risk of cardiovascular disease and HCC, and can progress to liver failure.

Treatment goals in NAFLD should be to stop the progression of liver disease, to manage metabolic risk, and to minimize cardiovascular risk. The treatment options of NAFLD include non-pharmacological therapy (lifestyle modification), pharmacological therapy, and metabolic surgery.

General measures for all patients

Abstaining from alcohol: Patients are advised to abstain from alcohol or avoid heavy alcohol use (i.e., >14 drinks per week or >4 drinks per day for men, and >7 drinks per week or >3 drinks per day for women) since heavy alcohol use is associated with disease progression (7).

Immunizations: Patients without evidence of serological immunity should be vaccinated for hepatitis A virus and hepatitis B virus. Additional vaccines recommended for patients with chronic liver disease are the pneumococcal vaccine and standard immunizations applied to the general population, including influenza, diphtheria, and tetanus boosters (8).

Weight loss: The best method that can be recommended to all patients at all stages of the disease is lifestyle modification. The primary treatment for patients with NAFLD is weight loss. Weight loss is recommended for all overweight patients with a body mass index (BMI) of >25 kg/m^2 or obese patients with a BMI of >30 kg/m^2 since it has been observed that weight loss improves the liver function test results and liver histology of NAFLD patients (9-12). A 5-7% weight loss should be planned for all patients that are overweight or obese, and the weekly target should be 0.5-1.0 kg loss. Patients with a diagnosis of NASH confirmed by a biopsy should lose 7-10% of their body weight. For some patients, weight loss may be required beyond these initial goals. If the serum alanine aminotransferase (ALT) level does not return to normal (<20 for women and <30 for men) after reaching the weight loss goal, patients are advised to lose additional weight. Although many studies have reported that patients need to lose at least 5% of their body weight in order to recover from hepatitis steatosis, the long-term effects of weight loss are not yet known. In a meta-analysis conducted with 373 patients, hepatic steatosis was seen to improve following a 5%

loss in body weight, and there was improvement in the NAFLD activity score with a 7% weight loss (13). In another study including 31 overweight and obese patients (BMI 25 to 40 kg/m^2) with biopsy-proven NASH, enrollment in a weight loss and exercise program was shown to result in greater weight loss after one year compared to a structured education program (loss of 9% versus 0.2% of body weight) (10). It was observed that patients in the weight loss and exercise group had higher rates of histological improvement compared to the education group (72% versus 30%).

In order to achieve a healthy weight loss, a personalized nutritional program, to which the patient can adapt, should be recommended in accordance with their physical, social and health characteristics, and daily calorie intake should be reduced by 500-1,000 kcal (14). Excessive intake of fructose, as well as the consumption of processed foods and diet drinks should be avoided. Daily fiber intake should be increased to 20-40 g/day. Twenty percent of daily calorie needs should be provided by proteins and 20-35% by fats. Saturated fats should be less than 10% of the daily calorie requirement and contain 5-10% polyunsaturated fatty acid and 15-20% monounsaturated fatty acid while trans fatty acids should not constitute more than 1% of daily calorie intake. For carbohydrate needs, complex carbohydrates should be prioritized, and refined sugar should be avoided (14,15). Coffee has been shown to have a protective effect on NAFLD (14); however, foods that are considered to have antioxidant properties, such as cinnamon and artichoke are not specifically recommended since there are no studies proving their benefit for patients with NAFLD.

Physical activity: It is known that progression of the disease and comorbidity risks increase in NAFLD cases with physical inactivity. It is recommended to increase the physical activity of

the person through at least 150 minutes of aerobic exercise per week and 20 minutes of resistance exercise (pushing, pulling, and lifting) three times a week (16).

Bariatric surgery: In NAFLD cases with a BMI of >40 kg/m² and NASH cases with a BMI of >35 kg/m² (without decompensated cirrhosis), if the weight loss goal is not achieved after six months of lifestyle interventions, including two nutritional counseling visits and if there is no response to pharmacological therapy, bariatric surgery should be considered as a treatment option to reduce weight and prevent metabolic complications. Bariatric surgery is a promising approach for obese patients with NAFLD, and postoperative histological improvement has been observed in several studies (17-19).

Pharmacological therapy

Pharmacological therapy can be used to promote weight loss in patients who cannot achieve their goals through diet and exercise alone. The recommendations for the use of drug therapy to promote weight loss widely vary among clinicians. Some clinicians do not use medication frequently while others prescribe medication to selected patients after providing comprehensive counseling on lifestyle modification measures.

Although there is not yet a specific pharmacological treatment for NAFLD and NASH proven to be effective in humans by randomized controlled trials, some agents have been investigated in this area and provided benefits at various stages of the course of these two diseases.

Pioglitazone: This is primarily used in the treatment of insulin resistance. Although the initial treatment of type 2 diabetes is metformin, which has no effect on liver histology in NAFLD (20,21), pioglitazone should be the second choice for diabetic patients with NASH if metformin is contraindicated or an additional treatment is required. In a study evaluating non-diabet-

ics, pioglitazone was used at a dose of 30 mg/day for up to 96 weeks and was observed to significantly reduce AST and ALT levels, hepatic steatosis, and lobular inflammation; however, it did not result in any improvement in fibrosis scores (22). In a meta-analysis published in JAMA, covering eight randomized controlled studies on the use of thiazolidinedione group drugs in NASH, the histological data obtained from the liver biopsy of a total of 516 patients followed up for six to 24 months were evaluated and the efficacy of pioglitazone and rosiglitazone was compared (23). It was reported that the use of pioglitazone resolved fibrosis in NASH even in non-diabetic patients. In a study published in Diabetes Care, all drugs used in NAFLD patients with diabetes steatosis, (80 and 120 mg elafibranor, obeticholic acid (OCA), liraglutide, vitamin E, and 30 and 45 mg pioglitazone) were compared in terms of their effects on six parameters, namely the NAFLD activity score, NASH resolution, lobular inflammation, ballooning, and fibrosis (24). As a result of these analyzes, although many drugs were found to have an effect on all parameters except for fibrosis, only 45 mg of pioglitazone treatment was also effective in fibrosis. Long-term pioglitazone therapy is required to achieve clinically significant benefits since improvement findings have been observed to regress upon the discontinuation of drug therapy. Finally, due to the large number of side effects of pioglitazone, such as bladder cancer, heart failure, anemia, and risk of fractures, it is only indicated for use in the presence of concomitant type 2 diabetes.

Vitamin E: For patients with biopsy-proven NASH or stage 2 fibrosis without a diabetes diagnosis, 800 U of vitamin E is recommended per day. Since studies on the benefits of vitamin E do not include diabetic cases and patients with decompensated cirrhosis, its use is not recommended in this group of patients. This is also in line with the recommendations of the American

Association for the Study of Liver Diseases (AASLD) (25). In the largest randomized controlled trial investigating the use of pioglitazone, vitamin E, and a placebo in 247 non-diabetic patients with NASH, the benefit of vitamin E was demonstrated. It was shown that when used at a daily dose of 800 U for up to 96 weeks, vitamin E caused a significant decrease in hepatosteatosis, lobular inflammation, and AST-ALT levels in NASH, but did not improve fibrosis scores (22). This effect of vitamin E is considered to be due to its antioxidant properties. In a meta-analysis, although there were differences between the five studies reviewed in terms of the formulation of vitamin E, diversity of the patient population, treatment duration, and lifestyle modification applied, it was reported that the use of vitamin E did not provide histological benefits (26). Considering that the daily need for vitamin E is 30 U, there are some analyses showing that using such high doses (800 U) for a long time can lead to an increase in mortality for all reasons, as well as increasing the risk of hemorrhagic stroke and prostate cancer (27-31). For this reason, this increased daily dose is not recommended for male patients with a personal or family history of prostate cancer, patients using multiple medications concurrently due to their comorbidities, and those diagnosed with diabetes.

Metformin: Although there are several small studies revealing that metformin decreases enzyme levels, no research has shown that it decreases both liver histology and NASH progression (32). In the TONIC study, in which vitamin E and metformin were compared in children and adolescents, unlike vitamin E, metformin did not have a positive effect on liver histology, except for aminotransferases and balloon degeneration (33). In conclusion, guidelines do not recommend metformin as a primary drug in the treatment of NAFLD/NASH, but it is reported that it can be used in supportive therapy (34-36).

Omega 3: This supplement can be used in hypertriglyceridemia accompanying NAFLD. Although histological improvement in NASH progression could not be demonstrated, in a meta-analysis of nine studies evaluating a total of 355 patients, it was observed that omega-3 treatment improved hepatic steatosis and AST levels (37).

Ursodeoxycholic acid (UDCA): This is used in the treatment of different hepatobiliary diseases to disrupt the vicious cycle of apoptosis, inflammation and fibrosis formed in the liver through different mechanisms (38). Due to its hepatobiliary cytoprotective activity and effects on cholesterol metabolism, researchers have considered that UDCA can positively affect the pathogenesis of NAFLD/NASH. However, as in metformin, although randomized controlled studies have shown that UDCA results in a decrease in aminotransferases, it is reported to have no effect on liver histology. Therefore, it is not recommended for the treatment of NASH in the joint guidelines of AASLD, the European Association for the Study of the Liver, the European Foundation for the Study of Diabetes, and the European Association for the Study of Obesity (39).

OCA: Although UDCA was found to be insufficient in the treatment of NASH, it has laid the groundwork for the development of OCA, which is a new derivative of the natural bile acid chenodeoxycholic (6-ethylchenodeoxycholic) acid. OCA acts as an agonist of the nuclear farnesoid x receptor (FXR), being 100 times stronger than UDCA. FXR activation in the small intestine reduces inflammation in adipose tissue and causes a decrease in free fatty acid flow to the liver. It reduces peripheral and hepatic insulin resistance by facilitating brown transformation and obesity in adipose tissue. With the decrease in fatty acid synthesis and increase in beta oxidation, the liver can allow inflammation to heal (40,41). In a phase 2b FLINT study, it was shown that OCA

provided improvement in all stages, including fibrosis in non-cirrhotic NASH patients. Phase 3 studies are ongoing. Since bile acid synthesis, which is the main degradation pathway of cholesterol, is suppressed, an increase in the LDL level leads to a decrease in the HDL level, raising concerns in relation to the potential cardiovascular risk that could be caused by long-term OCA treatment. Another undesirable side effect is pruritus, which develops in 25% of patients due to impaired enterohepatic bile acid cycle, and therefore it is recommended to start OCA treatment at low doses (42).

Statins-Fibrates: While statins reduce the cardiovascular risk in NAFLD, fibrates can be used as an adjunct to treatment in hypertriglyceridemia. However, neither group has been shown to have a positive effect on NASH progression. In some pilot studies, patients with NAFLD were reported to benefit from atorvastatin based on aminotransferase levels [43,44]. The use of atorvastatin use was subsequently examined in a secondary analysis of a study investigating the effect of atorvastatin, vitamin C, and vitamin E on the development of cardiovascular events in healthy adults [45]. The study had two exclusion criteria: a diabetes diagnosis and a serum aminotransferase level of >1.5 times greater than the normal upper limit. At baseline, 80 patients had NAFLD according to the imaging criteria. After a mean follow-up of 3.6 years, fewer patients in the treatment arm had NAFLD compared to the placebo arm (34% versus 70%, adjusted OR: 0.36, 95% CI: 0.16-0.83). However, the results that could be drawn from this study were limited due to the patients taking atorvastatin in combination with vitamins E and C, and the diagnosis of NAFLD being based on imaging criteria rather than histological findings. Furthermore, the exclusion criteria of diabetes and high aminotransferase level limited the generalizability of the data obtained.

Orlistat: This can be chosen as an auxiliary agent in patients with NAFLD + indigestion. It does not reduce the progression of NASH (46).

Liraglutide: In a study conducted with 52 patients diagnosed with NASH, 1.8 mg/day liraglutide treatment was applied for 48 weeks, and a liver biopsy was performed in 23 patients using liraglutide and 22 individuals forming the placebo group (47). While the NASH findings regressed in nine patients (39%) in the liraglutide arm, a similar regression was observed in two patients (9%) in the placebo arm. In brief, although liraglutide has no effect on fibrosis in NASH, it has been observed that it reduces steatosis and inflammation. It has positive effects on hepatosteatosis in proportion to weight loss in NASH patients with diabetes.

New Treatment Agents on the Horizon

Peroxisome proliferator-activated receptor (PPAR)-α/δ agonist: PPAR is a member of the nuclear receptor family. PPAR-α provides fatty acid oxidation by regulating lipid and lipoprotein metabolism enzymes; PPAR-γ is responsible for the storage of triglycerides in the liver and increasing insulin sensitivity, and has an anti-inflammatory effect; and PPAR-δ is involved in the regulation of fatty acid oxidation, mitochondrial functions, and insulin sensitivity. PPAR-α/δ agonists currently being developed in the treatment of NAFLD-NASH are considered to be both more effective and more appropriate in terms of their fewer side effects. Currently, the phase 2b studies of ***GFT505*** **(GENFIT)** are being undertaken, and it has already been proven to improve hepatic inflammation in patients with an activity score of ≥4.

Stearoyl-CoA desaturase-1 (SCD-1) suppressor: SCD-1 is the main enzyme that regulates fatty acid metabolism in the liver. Through the suppression of SCD-1, triglyceride and fatty acid in the liver decrease as fatty acid synthesis decreases and fatty acid

beta oxidation increases. ***Aramchol*** **(Galmed)** is the acid conjugate of two neutral components: colic and arachidonic acid. In the ongoing phase 2b study of Aracho, its effects on hepatic inflammation and fibrosis are evaluated by non-invasive tests.

Simtuzumab: Lysyl oxidase-like 2, LOXL2 is an enzyme that provides resistance to destruction by strengthening the crosslink between collagens in the extracellular matrix. LOXL2 is increased in a fibrotic liver. ***Simtuzumab*** **(Gilead)** is a monoclonal antibody developed against LOXL2. After observing that simtuzumab reduced fibrosis in experimental models, a phase 2b study was started and is still ongoing.

Cenicriviroc: C-C chemokine receptors (CCR), wide range of immune cells, are expressed by monocytes, macrophages, hepatic Kupffer cells, and natural killer cells. ***Cenicriviroc*** **(Tobira)** was developed as an anti-viral agent for use in HIV treatment. There is an ongoing phase IIb study, in which the effects of cenicriviroc on HCC and fibrosis in NASH patients.

Other agents with ongoing phase 2 studies include **Selonsertib** (protein kinase inhibitor), **Genkyotex** (nikotinamid adenin dinucleotide phosphate oxidase 1/4 suppressor), **Emricasan** (pancaspase suppressor), **Gilead** (apoptosis-signal-regulating kinase 1 suppressor), **GR-MD-02, Galectin** (Galectin-3 suppressor), **Vismodegib** (Hedgehog signal suppressor) and **Rimonabant** (cannabinoid suppressor).

There is no specific pharmacological treatment that prevents, stops or reverses the development of NASH and has been proven to be 100% beneficial with randomized controlled studies. This is because many enzymes, receptors and different pathways are involved in the pathogenesis of NAFLD/NASH. Based on the continuing research in the area, it seems that in near future, NAFLD/NASH treatment will be possible with the application of therapies individualized for each patient.

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Chapter-2

DIABETES MELLITUS AND PREOPERATIVE EVALUATION

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Many endocrine and metabolic disorders significantly affect surgical outcomes and anesthetic strategies. Patients may present with an operative endocrinopathy, or, more commonly, have an endocrine disorder that makes surgical and anesthetic management difficult. To predict and prevent complications due to an endocrine disorder, the underlying conditions should be evaluated thoroughly in the preoperative period. One of these endocrine disorders is diabetes mellitus (DM), which is a chronic metabolic disease that threatens human health worldwide (1). DM is characterized by chronic hyperglycemia, multiple organ dysfunctions, and cardiovascular, neurological and renal complications (2). Patients with diabetes, who can be relatively asymptomatic compared to the non-diabetic population, need to be carefully evaluated preoperatively for various reasons, such as high risk of coronary heart disease and increased postoperative cardiovascular morbidity and mortality rates, as well as the risk of perioperative infections (3,4).

In patients with diabetes, in addition to general reasons, surgical procedures may be frequently required due to the development of complications and other factors, such as peripheral vascular diseases, diabetic foot, vitrectomy, cataract, and the requirement of an arteriovenous fistula opening for the treatment of end-stage renal failure.

An important aspect of preoperative management in DM is glycemic control. Unbalanced blood glucose levels may occur due to the complex interactions involved in the surgical procedure, type of anesthesia (general anesthesia is associated with larger metabolic abnormalities compared to epidural anesthesia), extent of surgery (cardiovascular bypass surgery results in significantly higher insulin resistance), and postoperative factors, such as sepsis, hyperalimentation, glucocorticoid use, impaired meal schedules, modified dietary intake, and vomiting. Surgery and

general anesthesia induce a neuroendocrine stress response by the release of counter-regulatory hormones, including epinephrine, glucagon, cortisol, and growth hormone, and inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-alpha. These neurohormonal changes result in metabolic abnormalities; e.g., insulin resistance, decreased peripheral glucose use, impaired insulin secretion, increased lipolysis, and protein catabolism, leading to hyperglycemia and even ketosis in some cases (5-9).

Anamnesis and history

The preoperative evaluation of any patient, including those with DM focuses on cardiopulmonary risk assessment and modification. DM is a disease characterized by abnormal carbohydrate metabolism that causes hyperglycemia, which impairs vasodilation and leads to vascular complications by inducing chronic proinflammatory, prothrombotic and proatherogenic states (10). Although DM potentially affects all tissues, the atherosclerotic, vascular and renal effects of the disease, such as the development of peripheral vascular disease, renal insufficiency, and cerebrovascular disease (CVD) constitute the most important issues for the clinician. Diabetic patients often have autonomic dysfunction, which manifests as orthostatic hypotension,beat-to-beat heart rate variability, and delayed gastric emptying. Coronary heart disease is much more common in individuals with diabetes than in the general population, and the risk of silent ischemia is also increased in diabetics (11,12). Therefore, it is important to evaluate cardiac risk in this patient group (4). Other associated conditions, such as hypertension, obesity, chronic kidney disease, CVD, and autonomic neuropathy need to be evaluated prior to surgery since they can complicate anesthesia and postoperative care.

The preoperative evaluation of all patients requires carefully obtaining their medical history and performing a physical exam-

ination. The key elements of the initial assessment are explained below.

- The type of diabetes should be determined considering that patients with type 1 diabetes mellitus (T1DM) are at a much higher risk of diabetic ketoacidosis and basal insulin must always be provided.
- The long-term complications of DM, including retinopathy, nephropathy, neuropathy, autonomic neuropathy, coronary heart disease, peripheral vascular disease, and hypertension should be identified.
- Basic glycemic controls should be undertaken, including the monitoring frequency, mean blood glucose levels, range of blood glucose levels, and glycated hemoglobin (HbA1c) levels.
- If present, the frequency and timing of hypoglycemia and patient awareness about this condition should be evaluated.
- A detailed history of diabetes treatment, including insulin type, dosage, and timing should be obtained.
- Other pharmacological treatments should be questioned, including drug type, dose, and specific timing. A detailed history of drug therapy is required, especially in the elderly with a high probability of polypharmacy. The treatment regimen of patients with diabetes (T1DM or T2DM), compliance, timing and frequency of drug administration, especially insulin and insulin secretagogues or their combination therapy, additional drugs used (indomethacin, non-selective beta blockers, or antibiotics; e.g., trimethoprim-sulfamethoxazole) and alcohol use should be questioned in detail. When antihyperglycemic agents, such as GLP-1 agonists, dipeptidyl-dipeptidase-4 inhibitors, thiazolidinediones, acarbose, metformin, sodi-

um glucose co-carrier 2 (SGLT2) inhibitors are combined with insulin and/or sulfonylureas (SU), they may lead to unexpected hypoglycemic events (13). The presence of renal or hepatic insufficiency should be investigated. Progressive kidney damage can lead to a reduction in the detoxification of insulin or sulfonylureas and its metabolites from the blood [i.e., glyburide (glibenclamide)] (14).

- If the patient has a history of previous post-bariatric surgery, planning should be made being aware of the risk of pre/postoperative hypoglycemia.
- The characteristics of the surgery should be known in advance, including when the patient should fast, type of surgery (major or minor), and timing and duration of the surgical procedure.
- The type of anesthesia (epidural or regional anesthesia versus general anesthesia) should be planned in advance.

Laboratory evaluation

A basic laboratory evaluation should include a baseline electrocardiogram (ECG), assessment of renal function (serum creatinine), measurement of HbA1c if not obtained within the previous three months, and determination of blood glucose. ECG abnormalities, such as abnormal Q waves suggestive of previous myocardial infarction and chronic kidney disease are risk factors of important postoperative cardiac events. Further investigation, including non-invasive cardiac testing should be considered on an individual basis.

The HbA1c level should be measured if not previously evaluated within the last three months. HbA1c levels will allow the determination of chronic glycemic control, which is an important element in determining the adequacy of current glycemic management, particularly insulin dose in patients requiring insulin. Some researchers suggest that increased HbA1c levels predict

a higher rate of postoperative side effects, including infections, myocardial infarction, and mortality (15-18).

The goals of preoperative diabetes management include prevention of hypoglycemia and ketoacidosis/hyperosmolar conditions, maintenance of fluid and electrolyte balance, and prevention of marked hyperglycemia.

Hypoglycemia is a potentially life-threatening complication of poor perioperative metabolic control. Even for short periods of time, severe hypoglycemia [i.e., serum glucose concentration < 40 mg/dL (2.2 mmol/L)] can induce arrhythmias, other cardiac events, or transient cognitive deficits. Detecting hypoglycemia followed by neuroglycopenia can be difficult in sedated or anesthetized patients.

Patients with T1DM have an absolute insulin deficiency and tend to develop ketosis and acidosis. For T1DM patients, failure to provide pre-meal fast-acting insulin will result in unacceptable post-meal fluctuations in glucose. T2DM cases are susceptible to developing a hyperosmolar hyperglycemic state (also known as the non-ketotic hyperosmolar state), which can lead to severe volume reduction and neurological complications, and ketoacidosis may develop in this state of extreme stress. In addition, hyperglycemia can cause volume and electrolyte impairments mediated by osmotic diuresis and lead to calorie and protein loss in insufficiently insulinized patients.

Diabetic patients are also more susceptible to infection in the postoperative period. Observational studies show the presence of a relationship between preoperative or perioperative hyperglycemia and an increased risk of postoperative infection in this patient group (15,19).

Apart from the prevention of marked hyperglycemia and hypoglycemia, the optimal perioperative glucose targets are not clear. Different views exist concerning what the target blood glu-

cose should be, but there is only little evidence to support specific goals. Given the risk of hypoglycemia, the targeted glucose measurement should be 110 to 180 mg/dL (6.1 to 10 mmol/L). In a meta-analysis of 12 randomized trials (1,403 diabetic patients) comparing intensive [<120 or <150 mg/dL (<6.7 or <8.3 mmol/L)] and conventional (variable) glycemic control during the perioperative period, intensive perioperative glycemic control was observed to be not associated with a reduction in infectious complications, cardiovascular events, or mortality, but it was related to an increased risk of hypoglycemia (20).

Diabetes guidelines recommend glycemic targets of 110 to 180 mg / dL (6.1 to 10 mmol/L) for critically ill hospitalized patients (21,22). However, a less stringent glucose target [<200 mg/dL (11 mmol/L)] may be considered in patients with the risk of hypoglycemia, as well as potentially in the general patient population (assuming there is no evidence to support stricter goals). The risk of hypoglycemia can be reduced through frequent glucose monitoring and carefully designed management protocols. The American Diabetes Association has approved a target glucose range of 80 to 180 mg/dL (4.4 to 10 mmol/L) for the perioperative period (23).

In the preoperative evaluation of DM patients, ECG is useful in determining the presence of ischemic heart disease and providing a basis for comparison. Further stress testing may be necessary in symptomatic and asymptomatic patients that may have 'silent ischemia'.

Surgical preparation

Establishing simple and safe protocols in the preoperative evaluation of diabetic patients is essential for regulating blood glucose levels during and after surgery (Table 1) (24).

Table 1. Surgical preparation protocol for patients with type 1 and type 2 diabetes

1. The patient should be evaluated with A1C and PG measurements at least three to four days before surgery. Patients with insufficient glycemic control scheduled to undergo major surgery should be hospitalized two days before surgery, if possible. 2. High A1C is an indicator for inadequate metabolic control but is not a criterion for delaying surgery. However, surgery should be postponed in patients with metabolic decompensation and/or a PG value of >250 mg/dl. 3. An anesthetist and if necessary a cardiologist should be consulted. 4. If possible, surgery should be planned to be performed under elective conditions and in morning hours. 5. For patients that will undergo elective surgery, modified treatment should be applied from one night before considering that they will be required to fast on the morning of surgery.

In diabetic patients, pre-surgical treatment planning is generally undertaken by paying attention to the following rules:

- Generally, T2DM cases that are managed based on dietary modification alone do not need any perioperative treatment. Short (regular)- or fast-acting (lispro, aspart or glulisine) insulin can be given to patients with blood glucose levels exceeding the desired target. In this setting, insulin is typically administered every six hours. Blood sugar levels should be checked before and immediately after surgery. For long surgical procedures (lasting more than two hours) or those associated with expected high glucose levels (e.g., coronary artery by-pass grafting and steroid-used organ transplants), intraoperative glucose testing should be performed every one to two hours by laboratory or point-of-care testing. Venous or arterial blood and laboratory tests should be used in hypotensive patients or in cases requiring the use of vasopressor agents since fingertip glucose levels are less reliable in these groups (25).

- If DM patients using oral anti-diabetic drugs are to undergo

minor surgical interventions (those that can be performed under local anesthesia, those in which three cavities are not opened, and those in which oral nutrition can be started within a few hours after surgery), blood glucose measurements should be monitored every two hours on the operative day, and the anesthesia team should be informed that fluid containing dextrose should not be given during surgery. The patient, fasting in the morning of surgery, should be allowed to continue taking his/her medicine with the first meal after surgery.

- If major surgery is to be performed in DM patients using oral anti-diabetic drugs, oral anti-diabetic drugs should not be given to the patient on the morning of surgery, and the blood glucose level should be monitored to intervene with insulin when necessary. In order to reduce the risk of hypoglycemia, a few days before surgery, new short-acting agents can be used instead of SU group drugs with a longer duration of effect. In patients using metformin and SGLT2-I, it is recommended to discontinue these drugs at least 24 (48 hours if possible) before surgery and to provide adequate hydration. Routine treatment can be started with the meal after the procedure if the patient is able to orally take in at least 50% of his/her daily calories, if there is no acute renal failure, if contrast material is not planned to be given, and if the patient is planned to be discharged within 24-48 hours. Patients with uncontrolled diabetes should be prepared for surgery with insulin treatment in the preoperative period.

- If a minor surgical procedure is to be performed in patients with T1DM or T2DM using insulin and if the procedure is planned to take a short time, routine subcutaneous insulin treatment can be continued (26-28). Provided that plasma glucose is <200 mg/dl, if a procedure is planned in which only breakfast will be skipped in the morning, the patient should not take the short-acting insulin he/she normally takes in the morning. If plas-

ma glucose is >200 mg/dl, half of the dose normally taken in the morning should be given subcutaneously. In long-term procedures, short-acting insulin should be discontinued, and the dose of medium-acting insulin should be reduced by 50% on the night before. If the patient has a low risk of hypoglycemia and the dose is not too high, the long-acting insulin dose may be left the same. If risks are present, the dose taken on the previous night of surgery is reduced to half.

- If major surgery is to be performed in patients with T1DM or T2DM using insulin, the patient should generally fast from the morning of surgery and continuous glucose and insulin infusion should be applied until the procedure. Glucose and insulin infusions reduce metabolic disorders during surgery and increase the operative success. In the postoperative period, the infusion is continued until the patient can feed orally, then routine treatment is started. If the infusion is to be continued for more than 24 hours, Na + and K + control should be undertaken. In T1DM patients with an insulin pump, the basal insulin rate can be reduced by 25-50%, and the pump application can be continued during the operation. People with T1DM have an absolute insulin deficiency and should use insulin even if they are not hyperglycemic. However, in daily practice, having a device on the patient in the operating room environment is not preferred by the operating room team.

In the perioperative period, glucose and insulin can be administered by glucose-insulin-potassium (GIP) infusion or individual administration of glucose and insulin. There are numerous IV insulin infusion algorithms in the literature, in which insulin and glucose solutions are infused separately or as a combined GIP solution (26-29). Although the application of GIP is an easier method, individual administration may be preferred during major surgical procedures that may take a long time. Therefore, the

method to be chosen depends on the patient's condition and the experience of the team that will apply it.

GIP infusion

The GIP solution is a single-solution infusion containing 500 mL 5% dextrose, 10 mmol potassium chloride, and 10 units of short-acting (regular) insulin. The solution is infused at an initial rate of 100 mL/hour and can be modified depending on blood sugar by adding or removing five units of insulin. Potassium is added to prevent hypokalemia, and the levels are monitored at six-hour intervals. This regimen is safe because it involves the administration of insulin and glucose together, but the intravenous solution needs to be changed every five hours. The adjustment of the infusion rate according to the glycemia level is given in Table 2 (30).

Table 2. Adjustment of the infusion rate according to the glycemia level

Glycemia (mg/dl)	GIP infusion rate (ml/h)
≥280	140
279-220	120
219-180	100
179-120	80
119-80	60
<80	Infusion is suspended for two hours.

Individual administration of insulin and glucose intravenous solutions

In this regimen, dextrose is administered at approximately 5 to 10 g glucose per hour, and a separate insulin infusion is given using a short-acting agent. While most T1DM patients need an infusion of one to two units/hour, more insulin-resistant T2DM

patients may require higher insulin rates.

A commonly followed algorithm calculates the baseline rate by dividing the blood glucose level (mg/dL) by 100 and then rounding the result to units/hour (e.g., glucose level: 210 mg/dL, 210 / 100 = 2.1 units/hour) (27). Insulin infusion should be adjusted depending on fingertip glucose levels (e.g., a 0.5 units/hour increase for a glucose level of 120-160 mg/dL, 1.0 units/hour increase for 160-200 mg/DL, and 2.0 units/hour increase for >200 mg/dL). In case of hypoglycemia, insulin infusion can be reduced to 0.5 units/hour, and the glucose infusion rate can be increased to maintain the glucose target. The insulin infusion rate can be titrated depending on the procedure and degree of insulin resistance. This regimen is flexible, and in contrast to GIP infusion, it does not require all solution bags to be replaced.

There are several strategies to maintain target glucose levels during surgery, but there is no consensus on the optimal strategy (31,32). Most protocols for insulin administration are formulated based on expert opinion and personal experience. While the strategies described above are reasonable, they have not been proven to optimally reduce the morbidity, mortality, and consequences of length of hospital stay. The role of insulin infusions has not yet been clarified, but these strategies are often expensive, labor-intensive, and even impossible in some hospitals. Ultimately, even well-coordinated plans for diabetic treatment are dynamic and predictable, and sometimes affected by unpredictable events. Decisions concerning which regimens to use and when depend on patients, hospital settings and resources, and the clinician's own discretion.

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Chapter-3

USAGE AREAS OF ZIRCONIUM AND ZIRCONIUM IMPLANTS IN DENTISTRY

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Implantation refers to inanimate materials placed inside the body and living tissues. Dental implant is an artificial tooth root placed in the jaw bone and made of suitable material to restore the function and aesthetics of missing teeth; it is an issue studied from past to present (1). Expectations for the application of implant-supported prostheses have increased over time and nowadays implant-supported restorations have gained importance in single tooth deficiency. Dental implants are a type of crown (porcelain or zirconium) implant made by combining with a small titanium screw and abutment that is fully compatible with the human body (2). Zirconium (Y-TZP) is preferred today because of its use and durability, resistance to corrosion and aesthetic expectations. Zirconium, a highly reactive substance, is immediately coated with oxide in air and liquid and is resistant to corrosion. In order to meet aesthetic expectations, zirconium is more useful in front teeth than titanium. Because of the color of zirconium, it is used as an infrastructure material in aesthetic dentistry applications. Because zirconium teeth pass light, it creates an aesthetic appearance very similar to the natural tooth structure. Even well-supported metal-backed porcelain has a dullness and artificiality. Therefore, zirconium is preferred especially in the anterior teeth (3). In this sense, the implant material must have sufficient mechanical resistance, aesthetic appearance and biocompatibility to meet the needs of the patient and provide osseointegration with the tooth. osseointegration is defined as the structural-functional connection between living bone tissue and implant (4); biocompatibility, on the other hand, means the chemical interaction of materials and body fluids, and how much the physiological consequences of this interaction harm the body. In order for a material to be biocompatible, it must be accepted by the physiological environment in its living creature (5,6).

Zirconium implants, The use of zirconium as an implant body

material has been on the agenda in recent years(7). Reasons for using it as an implant body material; biocompatibility, chemical and dimensional stability, high flexural strength (900-1200MPa), adequate hardness (1200 Vickers), tooth-like color properties, low thermal conductivity, easy machinability, bone integration with titanium comparability, reduced plaque affinity and low corrosion potential (8-13). A positive biological reaction to zirconium was observed in cell culture studies(14) and animal experiments reported that osseointegration of zirconium implants was successful (15). Kohal et al. (15) compared titanium with zirconium implant loading in the same monkey models. No implant loss was found during the 14-month observation period and no mechanical problems were reported. In histological examination, there was no difference in the response of bone tissue between titanium and zirconium implants. The long-term success of the implant is directly related to the bone integration success of the material (16).

However, stresses in the implant material and the bone tissues surrounding it are another important factors affecting success. Caglar et al. (17). In their finite element stress analysis study, the stress of one-piece titanium implant, zirconium abutment screwed on a titanium implant and one-piece zirconium implants on the cortical bone was evaluated and reported that one-piece zirconium implants create less stress in the cortical bone. Due to concerns about the physical properties of zirconium implants, zirconium implants are manufactured in one piece. However, the greatest disadvantage is that the errors due to the angular misplacement of the implant during surgery in one-piece implants cannot be corrected at the prosthetic stage(18). If the implant is broken from any point, it cannot be repaired and must be removed from the bone(19). In recent years, two pieces of zirconium implants have been produced and presented for clinical use.

Most of the studies on this subject are case reports(20-22). Although it is not used in routine practice today, it is foreseen that zirconium implants will be applied especially in anterior region aesthetic cases in the following years(23).

The Development of Ceramic Implants improves the quality of life for many patients(24). Dental implants with many different forms, materials, and different surface properties are available on the market, but today commercial pure titanium implants with an in-bone cylindrical screw surface are considered the gold standard. The material frequently used in the production of ceramic dental implants today is tetragonal zirconium polycrystalline (Y-TZP, zirconium) stabilized with or without ytria in small proportions(25). The first to report ceramic implants (CBS: the Crystalline Bone Screw) is Sandhaus. In an average of 5 years, Crystalline Bone Screw only achieved 25% success(26).

In 1987, Sandhaus produced Cerasand (Incermed, Lausanne, Switzerland) ceramic implant, but there is no long-term clinical data on this system. In 1976, Schulte and Heimke introduced the aluminum oxide Tübingen implant (Frialit I; Friadent), which is used to implant anterior region. Besides the clinical reports of this implant, long-term scientific data is available(25). Alumina is known to be prone to fracture due to its low fracture resistance and therefore there are cases of implant loss in the maxillary posterior region. Therefore, the search for ceramic material to replace alumina as an oral implant has started. The ceramic material used in dentistry since the 1990s is zirconium(24,25). Ceramic implants were used as medical joints for the first time in the USA and Germany in the 1970s, and the Tübingen implant in the structure of Al2O3 was introduced in 1974 for dental endosseous implantation(26).

Despite being a biocompatible material, the high incidence of refraction caused titanium to replace this material(27). This

material has good chemical and physical properties such as low corrosion potential, low thermal conductivity, high bending resistance (900-1200 Mpa), durability (1200 Vickers) and Weibull module. Biocompatibility of Zirconium Implants Due to the specific crystalline structure, cracks caused by mechanical stresses do not cause implant fractures. In addition to high chemical resistance, bacterial uptake to zirconium implants is lower than titanium implants(28,29). Zirconium was used for the first time in animal studies as an oral implant coating material. In 1975, 5 of 9 zirconium-coated implants were surrounded by connective tissue and the results were not satisfactory in their study using a zirconium-coated Vitallium implant in dogs(26). In histological surface analysis of zirconium and titanium implants, Albrektsson et al. (30) in titanium implants, the proteoglycan layer, which was 20-40 nm thick, detected 30-50 nm thick in zirconium coated implants and Collagen fibrils found more in zirconium coated implants compared to titanium. In two studies, they emphasized the conclusion that zirconium as an implant material does not have a superior advantage over titanium. In various dog studies conducted in the early 1990s, the biocompatibility of alumina, zirconium and stainless steel was compared and it was shown that the affinity of different materials to bone was not different. However, researchers have reported that there is a thin fibrous membrane between the bone and the implant(31,32). Akagawa et al. evaluated zirconium implants loaded and unloaded one week after implant placement histologically after three months (33).

As a result of the study, the researchers reported that the implants were not mobile and there were no fractures during the experiment. Direct bone junction in the implant was evaluated for both groups and a week later, it was shown that there was more marginal bone loss in the loaded group than in the non-loaded group. In the study, no comparison was made with the titanium

control group. Although they are not used routinely clinically today, there is growing interest in zirconium implants. When the results of the recent studies are examined, the integration of zirconium with bone has become more compatible and it has been found that it is not different from titanium (34). Zirconium was evaluated in vitro with different cell lines such as fibroblasts, lymphocytes, monocytes, macrophages and osteoblasts. Zirconium powders (ZrO2 / Y2O3) have been shown to have no toxic effect on fibroblast cell lines. In biocompatibility tests on lymphocytes, monocytes and macrophages, Ca-PSZ powders and alumina were found to be less toxic than titanium oxide. (26)

On the other hand, reported that alumina particles showed high cytotoxicity in converting human monocytes into macrophages in their study comparing alumina and zirconium powders. In addition, zirconium has been reported to exert a cytotoxic effect on osteoblasts. Ca-PSZ and Y-PSZ did not show any local and systemic effects as a result of peritoneal injection in mice. Researchers assessing biocompatibility in hard tissues placed stabilized zirconium containing 6% Y2O3 in the monkey femur. It was stated that there was no growth, while no side effects were observed(35).

The microflora around the implants is similar to natural teeth and microbial pathogens (Actinobacillus actinomycetemcomitans, Porfiromonas gingivalis, Prevotella intermedia) associated with periodontitis can cause implant loss. Bacterial uptake and colonization on titanium were evaluated in vivo and in vitro. The degree of bacterial involvement and colonization to titanium implants is associated with surface roughness. Surface irregularity facilitates plaque accumulation(36). In their study, Rimandini et al. evaluated bacterial uptake on titanium and zirconium, they showed that early bacterial colonization on zirconium was less than titanium. In another study early bacterial involvement

and colonization on zirconium were reported to be significantly less than on titanium. In the roughening of zirconium implants, only air abrasion is performed, since acid etching has no effect on zirconium. However, zirconium implants and pores were coated with aqueous zirconium powder, and the pores were fired during sintering and the nozzle structure was obtained(37).

Two new techniques have been developed for surface conditioning of zirconium. In the plasma spray technique, plasma atoms containing ions, electrons and neutral particles and an ionized gas are applied to the surface under vacuum. With this method, the binding force can be increased by covalent bonds. In the other technique, it is argued that the bonding can be increased by placing porcelain pearls on the inner surface of the ceramic(38). In the study evaluating the early dental plaque formation of glazed and polished Y-PRP, no difference was found between the two groups. However, it has been stated that more plaque accumulates on glazed surfaces due to irregularity on the surface. Zirconum ceramics tend to age (aging) despite their high durability. Although aging has detrimental effects on the mechanical properties of zirconium, resistance values have been reported to be clinically acceptable. Conversion spontaneously or slowly from the tetragonal phase to the monoclinic phase is called low heat disruption and can lead to changes in ceramics, reducing its durability. Corruption; temperature, steam, stress, particle size, micro and macro cracks of the material, concentration of stabilizing oxides are affected by production and veneer techniques. In order to prevent this, different stabilizing oxides, applied fabrication techniques and protocols need to be changed (39,40).

In a study, the molecular response of the titanium-zirconium and commercially pure titanium implants was investigated and the degree of osseointegration of the implants was compared biomechanically. They suggested that there was a strong interconnection

between implant surfaces and bone, where both materials were successfully osseointegrated and showed high Removal torque (RTQ) values after 12 weeks. However, another study has suggested that healing kinetics are different for two implants (41-43).

In a study, RTQ results confirmed previous results. RTQ values were quite low at the beginning in both groups. It is believed that such a result is probably due to the harmony of rabbit tibia, which consists of a dense cortical layer of several millimeters around the bone marrow cavity(43). The values have increased significantly with the advancement of osseointegration and reported that it has reached an average of 135 Ncm, with consolidation around the implants, as shown in previous histological studies. At the 4th week, the force required to relax the implants was reported to be high for both groups(43). As the biggest innovation of the study, they stated that the two types of implants shed light on the molecular mechanisms that can lead to different healing kinetics. It has been reported that TiZr implants are the first to investigate the activation of bone genetic markers, Growth factors (RUNX2, IGF1, BMP2), extracellular matrix proteins (ALPL, COL1A1, BGLAP [osteocalcin], SPP1 (osteopontin)). After 2 weeks of recovery, a statistically significant arrangement of all genes screened in the tissue surrounding TiZr implants was observed. Among these, BMP2, ALPL, COL1A1, TNFSF11, CALCR, and IL6 values were stated to be at least two times higher than the control in the TiZr group(44). The BMP2 gene is a family of bone morphogenetic proteins (BMPs), one of the growth factors that have paracrine activity and play an important role in osteogenesis (45). Of these, BMP2 is considered an osteoinductive factor as it can induce ectopic bone formation (46). However, it is not clear whether his greatest contribution was involved in the recruitment or differentiation of mesenchymal stem cells after reaching the site for repair. In all cases, BMP2

has been reported to be mandatory for bone regeneration following fracture healing (47,48) and for implant healing as claimed (49,50). BMP2 expression was found 2.17 times higher in the tissue surrounding the TiZr implants, and it was emphasized that TiZr surfaces may suggest that it is conducive to bone regeneration. Explain why these implants have a significantly higher bone placement in response to the medullary segment (43).

Since the introduction of zirconium oxide ceramics in dental practice, the fields of use have been expanding with increasing speed. In-vitro and clinical studies also increase the interest of physicians on zirconium-supported materials and encourage their use in routine applications. In the following years, it may be the primary reason for preference in dental practice by revealing material-specific limitations and indications more clearly.

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Chapter-4

FACTORS AFFECTING THE PROGNOSIS AFTER BREAST-CONSERVING SURGERY FOR EARLY STAGE BREAST CARCINOMA: LOCAL RECURRENCE AND OVERALL SURVİVAL

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1. Introduction:

Breast cancer (BC) is the most common malignancy among female gender worldwide accounting for over two million cases each year , reaching the highest numbers in Western countries, especially in the United States (1). It is also the leading cause of cancer death in women worldwide. The mortality rates related to BC have been decreasing since 1999 due to the widespread and effective application of adjuvant systemic therapy (2). Breast conserving surgery (BCS) is defined as either complete excision of the tumor or total excision of a quadrant of breast with acceptable clear margins combined with sentinel lymph node biopsy of the axilla and nowadays has been considered as a first line surgical treatment option for early stage BC (3).

Unappropriate therapy protocols, either over-treatment or under-treatment depending upon the final pathology report may interfere with the success of disease-related prognosis, but many other factors including tumor biology and surgery related factors also affects local recurrence, distant metastases and overall survival of BC survivors (4). For early-stage BC, BCS can achieve an equivalent rate of overall survival compared to total mastectomy. An overall risk of 5% at 5 years was defined for local recurrence following the surgical excision of tumoral area to clear margins combined with appropriately planned and applied local radiotheray (5). Studies revealed that 25-30% of patients who had undergone BCS for early stage BC require reoperation in succeeding 10 years (6-7). None of these studies could be able to define a systematic and clear definition for prediction of outcome for individual patients in order to optimize the choice of surgical treatment modality. There is a lack of validation for established biological tumor parameters and

biomarkers to predict local recurrence in early-stage BC. Since it is well established that optimal local control of the disease reduces also BC-related overall mortality rates, a properly performed surgical procedure followed by irradiation of the whole breast with optimal dose with or without a cavity boost is the sine qua non of breast conserving treatment (1,3).

A prognostic factor is defined as a marker capable of providing information on clinical outcome of a specific disease at the time of diagnosis, independent of therapy. According to oncologic principles these markers are usually good indicators of growth, invasion, and metastatic potential of aforementioned tumoral processes. On the other hand predictive factors provide the physician a quite presumptive information on the likelihood of response to a given therapeutic modality. A prominent prognostic factor in BC should provide signıficant and independent value with readily interpretable results and should not consume pathological tissue needed for other tests.

Among women with early BC, the locoregional treatment control does not depend to BC subtype and better patient-tailored therapy decision involves a more complicated algorithm regarding risk of local recurrence and distant metastasis (3). Clinical utility of the risk factors likely to be responsible of unfavorable prognosis should be assessed in large number of patient series.

This review will focus on the prognostic and predictive factors of local recurrence, distant metastases and overall survival for early-stage BC survivors who had undergone BCS.

2. Local-Regional Recurrence:

After BCS either lumpectomy or partial mastectomy, followed by whole-breast radiation therapy (WBRT), BC can recur

locally or regionally correlated to both pathological and clinical several parameters. Local recurrence is defined as recurrence of tumorogenesis in ipsilateral preserved breast. On the other hand, a regional recurrence is nominated to tumor cells invading the ipsilateral regional lymph nodes, usually ipsilateral axillary, supraclavicular, infraclavicular and/or internal mammary. The term loco-regional recurrence (LRR) is dedicated to the recurrences occuring in either the ipsilateral preserved breast or regional nodal basin (8). In many studies, it is revealed that some patient and disease characteristics tend to affect the LRR rates so improving locoregional control is one of the main aim in patient-tailored therapies.

Either invasive or in situ cancer can be encountered in local recurrence after BCS. Patients who were initially treated for invasive BC regardless of molecular subtypes have been found to develop invasive type of BC recurrence in 80% of cases, the remainder of LRR are noninvasive (in-situ) lesions in which the tumor cells do not penetrate the basal membrane. Approximately 75 percent of cases are isolated local recurrences with clinically solitary tumoral lesions; only 5 to 15 % present with a simultaneous regional lymph node involvement, and another 5 to 15 % have distant metastases at the time of diagnosis either symptomatic or insidious (9-11). Conversely, for patients primarily diagnosed as in situ cancer (ductal carcinoma in situ [DCIS]) and treated as with its own treatment alghorithm, , half of the recurrences seems to recur with invasive disease, whereas the remainder will tend to recur in the form of de novo DCIS (12).

In-breast tumor recurrences (IBTRs) after BCS are mostly suspected by screening mammography alone and proven by histological examination, preferably by core biopsy of the most suspected area. If the is more than one suspected lesion, multipl biopsies may be needed fort he affected individual. Skin inlvolve-

ment and chest-wall recurrences are the situations complicating the choice of treatment modality so these cases should be evaluated by more sophisticated imaging modalities, including Magnetic Resonnance of the breast (MRI) (8).

2.1 Risk Factors for a local recurrence

Several factors including patient and disease characteristics have been associated with increased risk for LRR. Younger age at time of diagnosis, larger tumor size, close margin status, positive nodal status, higher tumor grade, extensive intraductal component, multifocal or multicentric disease, negative hormone receptor status, lymphovascular and perineural infiltration were the most commomly described factors which are important predictors of LRR. Research efforts have focused on how we can personalize the optimal treatment to improve local recurrence rates. This section will imply on all these factors accused to be a risky condition for LRR (9-12).

2.1.1.Younger age at time of diagnosis:

It has been known that young age at fist BC diagnosis is a risk factor following BCS. Especially patients diagnosed with BC earlier than 35 years of age have the highest rates of LRR. The threshold age varies from 32 years to 40 years according to the series. These patients should be evaluated very carefully and patients should be keep informed about the probable risks before deciding a BCS even if tumor size is suitable and other prognostic factors are favorable (3,9). Although young age is not an absolute contraindication for BCS, a more extensive and careful follow-up programs should be considered for these patient groups.

2.1.2.Larger tumor size:

Macroscopic tumor size (pT) was one of the primary factors taken into account for LRR. Large tumor size especially T3 tumors (tm size > 5 cm) is also a relative contraindication for BCS, both because of less acceptable cosmetic results and also micro-

scopic satellite lesions that can be underestimated by radiological imaging methods. Tm /breast size ratio is also an important factor in surgical treatment decision, since surgical clear margin achievement may need multiple re-excisions which render unvantable clinical conditions for both patients and clinicians. More than two re-excision of the same margin increases the risk of LRR two-fold, so the better tecnique for these cases are assumed to be total or subcutaneous mastectomy of the breast tissue (6-13)

2.1.3 Close tumor margin status:

The main goal for all patients undergoing BCS is a complete tumor excision with surgical negative margins preferably without more than re-excision for one site. For invasive BC, the goal should be to achieve a tumor-free microscopic pathological evaluation at the inked border at every part surrounding the tumoral tissue. A wider surgical margin is preferable for patients who have isolated DCIS or invasive tumor surrounded by DCIS, due to a greater propensity for multifocality, especially with some skipped areas between the tumoral tissue (14). BC patients who were treated with negative surgical excision margins (typically defined by the National Surgical Adjuvant Breast and Bowel Project [NSABP] as the absence of either invasive or intraductal disease at the inked margin) have been found to have lower rates of LRR compared with those who have involved margins. Smitt et al. revealed that the five year LRR-free rate after BCS was 100 percent among patients with clear surgical margins versus 78 percent for those without clear margins (15). Similarly Houssami et al, in their relatively up-to-date metaanalysis claimed that margin status has a prognostic effect in all women treated for invasive breast cancer; increasing the threshold distance for declaring negative margins is weakly associated with reduced odds of LRR (16). All these data sets a good example of the pri-

mary prognostic value of achieving tumor-free margins in final pathological examination. Either already positive or unknown tumor-free margins should be re-evaluated for prompt reexcision, since these patient group is well-known to set a higher risk for LRR even if sine qua non adjuvant radiotherapy is performed in a well-planned manner (14-16).

While the pathologic finding of negative margins without any evidence of invasive or in-situ tumor cells at the inked border of pathological specimen on microscopic examination optimally reduces the risk of LRR, there is no proven data that wider margin range additively protects the individuals from LRR. A metaanalysis from 21 trials, conducted by Houssami et al. with 14.571 women with early breast cancer treated with BCS ended up with 1026 women who developed a LRR. In this study, it is revealed that a positive margin or a margin less than 1 mm was found to be closely associated with increased risk of LRR, yet there was no a statistically significant difference in the range of LRR by the width of a negative margin attained (16).

In the light of these data derived from a systematic rewiev of the literatüre, a multidisciplinary consensus concluded that unnecessarly wide tumor-free margins on microscopic pathologic examination have no significant impact on the rate of LRR as well as these type of wide-margin surgeries have been found to be come out worse cosmetic results and less patient satisfaction (16-17).

2.1.4.Positive nodal status:

Over the last several years, many changes have occured in the management of the axilla in BC surgery. AMAROS and ACOZOG Z0011 trials have proposed that in case of low tumor burden in axillary region proven by sentinel lymph node biopsy (SLNB) simultaneous to the breast surgery may be ideally managed by irradiation of axilla with a similar recurrence rates as axillary dissection. The multicenter phase 3 trials enrolled with T1

and T2 BC patients assigned to axillary dissection versus SLNB+ radiation treatment focused on the reliability of irradiation for axillary recurrence (8).

The impact of axillary nodal status on local tumor control after BCS remains uncertain. Data on the risk of LRR as a first recurrence area in patient subgroups defined by the number of involved lymph nodes in the primary surgical pathology specimen are limited and somewhat confusing. As a long-standing trial, in the Danish trial, 30% of the patients with one to three involved lymph nodes, that means patients with pathological N1 in the primary surgery were found to develop LRR in consecutive 10 years whereas this rate was actually 16% in 10 years and 33% in 15 years in British Columbia trials. These designated values were substantially higher when compared to those in the few other published series with more than 5 years of follow-up that reported results according to the number of involved nodes (6% to 13% for 10 years) (18-19).

The nodal status affects primarily the overall survival that has been designated in many rewievs in literature search. Hereby for LRR, the main proven predictory factor is found to be the axillary involvement of the disease, not the number of the involved lymph nodes (13,16). In some studies, it has been shown that this relationship is also supported by the size histologic characteristics of the tumor, so it is possible to mention a parallel correlation in increase in LRR. To search for additional information on this subject, the factors related to the risk of LRR should be compared in a manner that the disease characteristics of the patients are similar. Arriagada et al. reported that in patients who had BCS enhanced with axillary dissection, the number of axillary lymph nodes examined was not predictive of LRR in any of the histological subgroup analyses, and the prognostic value of lymph node involvement should be rejected in patients with

small tumors in whom the risk of having positive axillary nodes was low. Another important finding of the study was that the number of positive axillary nodes was not a predictive factor for LRR in node positive patients. The limitation of this study was that all patients had undergone axillary dissection without performing sentinel lymph node biopsy, so there was a group of patient overtreated by axillary dissection (20).

It is noteworthy that lymph node involvement >7, that means N3 patients group has a special consideration in many studies. Especially small tumors with N3 status are found be highly succeptible to LRR after BSC which may be hypothesized by the molecular aggressivity of the tumor and extensive multicentricity of the tumor that could not be detected by the standard radiological imaging modalities (2,3,20).

2.1.5.Higher tumor grade:

The higher histological grade in BC has been demonstrated to be associated with LRR in mastectomized patients in previous studies. Although some studies noticed that there can be a positive correlation between higher tumor grade and increased LRR after BCS, none of the studies had demonstrated a difference between mastectomized and BCS patients in terms of LRR (20-22) It is possible to refer to a general effect but studies with large number of patients with different molecular sub-type tumors would be required to attain statistical significance.

2.1.6. Extensive intraductal component (EIC):

EIC is the term referred to infiltrating ductal tumoral involvement in which more than 25% of the whole tumor volume is consisted of DCIS. It is noticed that in BC presented with EIC, DCIS seems to extend beyond the invasive tumor into the surrounding breast tissue which is described nearly innocent with most sensitive radiological imaging methods even with MRI. The only radiological sign may be the combination of a mass and associated

calcifications in the suspected patient group (8). Literature search revealed that 15-30% of BC patients' tumor is accompanied by EIC regardless of molecular sub-type (8,23-25). EIC can be a predictor for higher incidence of residual tumor (especially in the form of DCIS) following BCS even all the palpable tumoral tissue is excised with a pathologically proven clear margin. Per-operative mammographic examination of the excised tissue compared with previous mammogram in terms of clear margin may be an option to preserve the breast tissue and to avoid unnecessary mastectomies (23). Density of the corresponding breast tissue is an important factor that can limit the sensitivity of mammography both for delineating disease extent and deciding the extent of excision (22, 25-27)

The notion that EIC may have limited importance as a risk factor in post-menopausal patients was established in 1990's with some reports mentioning that younger patients tend to develop more EIC accompanying their primary tumor than patients older than 50 years of age. Not only menopause status but also estrogen receptor (ER) positivity is also closely related with age in EIC setting. As a classical literature finding, post-menopausal, ER (+) patients older than 50 years of age are less prone to develop LRR even if their BC is presented with EIC (28).

2.1.7. Negative Hormone Receptor Status:

Analyses of LRR by molecular subtype and hormone receptor status of the underlying tumor reveal that hormone status of the tumor may play a role in locoregional control, for example ER- and PR-negative BC, especially the basal subtype which remains an almost therapeutic challenge for clinicians, may also be associated with worse prognosis in terms of greater risk of LRR after BCS (8,22)

Adjuvant hormonotherapy for ER(+) patients and targeted therapies for HER2 (+) group seem to be effective in local con-

trol of disease after BCS. Many studies reported that HER-2 and basal subtypes demonstrated an increased risk of regional recurrence after BCS (21,22,29). In patients with ER(+) disease, there is strong evidence that adjuvant hormonotherapy reduce significantly LRR risk by 40% after follow-up of 15 years. The locoregional benefit was independent of PR status, age, nodal status, or use of adjuvant chemotherapy. On the other hand, the different patterns of recurrence among BC regarding the molecular subtypes could partially be explained by "early" versus "late" LRR. Luminal A and B (ER positive) tumors tend to have a more indolent course so the rates of LRR among the subtypes might equalize over time. Longer follow-up with larger and well-designed patient population will be required to make a statement about the pattern of LRR among BC subtypes (29).

2.1.8. Peritumoral vascular and lymphatic infiltration:

The presence of peritumoral vascular and lymphatic invasion (PVLI) was a predictor for isolated LRR as well for other recurrences. Although the absolute number of isolated LRR was higher especially in younger age group patients, PVLI has been considered as an independent risk factor in all ages (16,30). The prognostic significance of vascular invasion for LRR was independent of all other factors. Vascular invasion was found to follow age and lymph node status in predicting for LRR in BCS patients (30). Definite lymphatic infiltration of the tumor revealed an 57% of concurrent axillary metastasis which is another co-factor increasing LRR. Otherwise, some studies have suggested that lymphatic invasion was the most important prognostic factor even more significant than age, tumour size, grade and type especially in lymph node negative BC disease (30,31).

3. Disease-free (DFS) and overall survival (OS):

Accurate prediction of DFS and OS in a newly diagnosed BC patient is one of the most challenging burden that the physicians

encounter. Many studies have been focused on DFS and OS after BCS and proposed different prognostic factors determining also high risk group of patients to be treated and followed-up more intensively. Although the results obtained so far can not be considered optimal for many aspects, investigations over the factors affecting the survival and estimating the survival time of newly diagnosed early BC patients have confirmed the beneficial effects of a multidisciplinary approach of auxiliary treatment, including combination chemotherapy, radiotherapy and hormonotherapy in effective cases (2).

In recent studies, one of the most adressed issues among all the factors affecting both DFS and OS is the type of surgery conducted. Many studies revealed that the hazard of death of patients who had undergone BCS for BC is less than mastectomized patients, independent to other factors (33-36). In order to determine an accurate prediction of the clinical outcome in selected BCS patient groups, the paramount importance of reliable prognostic information provided by histopathologic and molecular mediators should be emphasized in each case separately. Moreover, sensitivity or resistance of tumor cells to the scheduled treatments, either cytotoxic or hormonotherapy, should be identified by clear predictors of tumor biology (2,32). So far the most extensively investigated predictor and prognostic factors for DFS and OS are age, stage, tumor size and axillary lymph node status at the time of diagnosis, estrogen and progesterone receptors (ER and PR), HER2 expression ,various growth factors, oncogenes, tumour suppressor genes, tumour angiogenesis factors and apoptosis-related factors.

3.1. LRR

Although patients with BCS received a guideline adherent treatment have been shown to have lower incidence of distant metastasis and higher expected survival time compared to mas-

tectomized patient group, LRR following BCS especially in the first three years, is considered an independent risk factor for shorter DFS, especially with bone and brain metastases (2,34). Although no statistically significant data has been reported about the effect on OS, unpublished data from ongoing studies show also an OS disadvantage in this patient group. NSABP-B06 (National Surgical Adjuvant Breast Project) study has somewhat similar results, in which BC patients with LRR following BCS were 3.41 times more likely to develop distant metastases, and patients with early LRR has been shown to develop metastasis than those who experienced late LRR (37). Although receiving post-operative WBRT reported to reduce LRR 3.4 times, there is no statistically significant data about the positive effect of WBRT over either DFS or OS. The findings of most of the studies targeting the relationship between LRR and systemic disease concluded that LRR can be accepted as a marker rather than a cause for progression to systemic disease (33).

3.2. Age at time of diagnosis:

As a predictory factor for LRR, age at the the time of diagnosis has also a negative correlation with DFS; higher risk of distant metastasis can be considered in any patient <40 years of age regardless the stage, size and subtype of tumor (31). These patients generally present at a later stage of the disease, and OS is approximately 74% in five years period. ER(-) and basal-like tumors with higher grade tend to ocur in younger age group so other unfavorable prognostic factors increase also higher risk of early distant metastasis and death (38). On the other hand, the main survival disadvantage appears in luminal group when compared the patients' ages; essencially young aged patients in luminal group had increased BC mortality rates compared to older patients (34,38).

Another important point is that BC patients>65 years of age have been demontrated to have higher mortality rates not only

increased risk of distant metastasis but also due to late diagnosis of the disease, higher comorbid factors and resistance to adjusted treatment modalities (37).

3.3. Stage, tumor size and axillary lymph node status at the time of diagnosis:

In all types of cancer, stage of the disease at first presentation is a prognostic factor, just as in case of BC. BC staging is validated by the tumor size, presence and number of the involved axillary lymph node, infra and supra-clavicular lymph node involvement, skin and chest wall involvement and presence of distant metastasis . According to American Joint Committee on Cancer (AJCC) eighth edition staging system, five-year DFS rates were 98-100%, 85-98%,70-95% for stage 1, 2 and 3 BC respectively (39).

Although tumor size at presentation is found to be directly related to lymph node status in all histological subtypes, it is considered as an independent prognostic factor for DFS and OS in BCS patients. Especially patients presented with T3 and T4 tumor, tumor size>5 cm with skin or chest wall involvement, treated with BCS either as a primary treatment or after neoadjuvant chemotherapy have been found to be associated with a worse prognosis, shorter DFS and OS (34). Only in the triple negative (ER,PR and HER2 negative) group, the correlation between tumor size and nodal involvement is absolutely weak and yhe prognostic implication of tumor size is negligible (34,38).

The presence and the number of involved ipsilateral axillary lymph nodes appear strongly associated with prognosis with independent negative indicator. A recent study conducted by Siegel et al revealed that five-year survival rates of patients of N0 and N1 disease were 99% and 85% respectively, regardless of histological subtypes. Even if T1 tumors with nodal involvement have been regarded to have worse prognosis than larger size tumors without any lymph node involvement (40). According to the Na-

tional Surgical Adjuvant Breast and Bowel Project (NSABP) B-32 trial, occult lymph node metastasis, metastasis <2mm in diameter, was found to be an independent prognostic factor for LRR: however, it had no statistically significant relation to OS (41).

3.4. Histologic grade:

Histologic grading of BC is defined by the percentage of tubule formation, nuclear pleomorphism, and mitotic activity, which are systematic determinants of the degree of tumor differentiation (4). A positive correlation has been found between histologic grade and survival of patients, especially for the patients with grade 3 tumor who shows a significantly higher risk of distant metastasis development. The correlation persisted and consolidated by the larger tumor size and the presence of lymph node involvement (39).

3.5. Peritumoral lymphovascular invasion:

Lymphovascular invasion (LVI), defined as the overgrowth of tumor cells into the endothelial-lined lymphatics or blood vessels within the breast parenchyma surrounding the tumoral mass, have been reported to be associated with poor prognosis in BC patients. This prognostic significance is related to increased risk of axillary lymph node involvement and distant organ metastases (11,13). When LVI was stratified by other prognostic factors, especially with >5 cm and basal-like tumors with axillary involvement, DFS and OS rates were found to be significantly lower compared to group without LVI. Especially, BC presented with hormone receptor-negative biological behavior enhanced with LVI was found to be directly linked to higher risk of LRR and death. Although the prognostic mechanism of LVI has not been completely elucidated, a model of microinfiltration of the tumor by embolism of vessels may denote the aggressiveness and refractory to treatment characteristics of the lymphovascular tumor burden (34,38).

3.6. Ki67:

Ki67 is a nuclear nonhistone protein and considered as a proliferation biomarker to predict the risk of LRR and extent of chemotherapy benefits for patients who are candidates for neoadjuvant chemotherapy. As a marker found in all proliferating tumor cells and a consistent prognostic factor for early-stage BC, there is no elucidated optimal cut-off point and scoring protocol for Ki-67 expression as a predictive factor for DFS and OS (42). Despite heterogeneity in metaanalysis results, Ki-67 has been accepted as an independent prognostic marker in terms of DFS and OS in BC patients. The Ki-67 cut-off value >25 % has been regarded to carry a greater risk of survival disadvantage rather than lower expression rates.(36-38,42).

3.7. Receptor status:

3.7.1. Hormone receptors:

ER and PR expression are historically primarily defined good prognostic factors for BC in terms of LRR, DFS and OS. Especially ER positivity defines the adjuvant hormonotherapy convenience of the individual, which has preventive effects on both local recurrence and distant metastasis (3,7). Data from metaanalyses suggest that there is distinctive positive correlation between OS, DFS, and time to treatment and positive ER and PR status. On the other hand, although the annual rate of recurrence for ER (-) patients is higher in the first five years succeeding the initial treatment compared to ER (+) patients, in longer-term follow-up, specifically after five years,in other words after completion of hormonotherapy, patients with ER(+) disease has a steadily increasing risk of annual recurrence (41).

Hormonal status of tumor in BC is also shown to be associated with type of metastatic spread for target organs. For some reasons that can not be elucidated clearly, ER(+) tumors are more prone to metastasize to bone, soft tissue, or the reproductive/gen-

ital tracts; on the other hand, ER(-) tumors are more likely to invade vital organs including lung and brain, which decrease OS rates. ER(+) tumors are generally consisted of histologically well-differentiated tumor cells with a low dividing potential and mostly diploid in character. All these factors contribute to the gfood prognostic facility of estrogen gate in BC. Mutations, loss, or amplification of breast cancer-related genes such as p53, HER2, or HER1 (the epidermal growth factor receptor [EGFR] , all of which have been associated with a worse prognosis, are rarely seen in ER(+) BC patient group (43).

PR appears to be an independent prognostic factor even if ER is positive or negative. Not only its negativity but also low percentages of PR has also been associated with poor prognosis and resistance to treatment even if ER is positive. A large population-based cohort study consisted of more than 1000 women with early BC, all of whom underwent primary surgery with curative intent followed by adjuvant hormonotherapy demonstrated that PR (+) influence both DFS and OS positively (44). Absent PR expression was associated with poorer prognosis for OS and DFS, even within the ER(+), lymph node-negative group, and was not influenced by endocrine therapy. These data are supported by the finding that patients with ER(+), PR(-) disease have a more aggressive subtype of hormone receptor-positive breast cancer , and with a higher Ki 67 value mostly considered into the luminal B subtype of tumors (43,44).

3.7.2 HER2 Status:

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