40 CHAPTER

Patient Optimization before Regenerative Therapy

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INTRODUCTION

We often accompany different patients with a pathology at a very similar stage, and even though these individuals receive exactly the same treatment, one has a much better result than the other. In cancer, for example, often two patients are diagnosed with a specific type of the disease, at exactly the same stage of evolution. However, one of them languishes quickly while the other lives for years, seeming not to have been overcome by the disease. When we are dealing with candidates to receiving regenerative therapy, the same intriguing story takes place.

Medicine has found numerous factors that influence the human organism's ability to recover. Many of them are not able to be modified, such as age and genetics. On the other hand, factors such as lifestyle, diet, supplementation and quality of sleep are totally modifiable [1]. In regenerative medicine, we depend directly on the body's response to the treatment administered to the patients. In this context, an adequate cellular response relies on a number of factors, like the absence of chronic systemic inflammation, the functional capacity of the cells of the immune system and the presence of essential nutrients for the proper functioning of the body (vitamins and minerals).

In this chapter, there is no intention to discuss all aspects of patients' metabolic status. However, key points for metabolic optimization are presented. First, it will discuss some important semiological points in the evaluation of patients, followed by basic laboratory tests that are requested, before regenerative treatment are suggested. Finally, therapeutic options that may be individually evaluated according to the patients' needs are also discussed.

METABOLIC SYNDROME AND INFLAMMATION

The first investigation that is carried out on a likely candidate for regenerative treatment are the suggestive signs of metabolic syndrome. Scientific studies show that the presence of metabolic syndrome considerably decreases in the regenerative capacity of the progenitor cells and immune system [2].

Metabolic syndrome (MS) has become one of the major health burdens for over three decades not only due to its effects on cardiovascular system but also its implications in orthopedics. Extensive researches have shown that MS is tightly linked to osteoarthritis and inflammation. Obesity, dyslipidemia, insulin resistance and hypertension are the top metabolic risk factors. These factors are responsible for the

disruptive physiological processes that culminate in alterations of the subchondral bone, cartilage damage and, overall, the predominantly pro-inflammatory joint microenvironment [3].

The inflammatory state that accompanies the metabolic syndrome does not completely fit into the classical definition of acute or chronic inflammation, as it is not accompanied by infection or massive tissue injury. So it is often called 'low grade' chronic inflammation or 'meta-inflammation', meaning metabolically-triggered inflammation [1].

In this scenario, MS and type 2 diabetes contributes to decreased multipotency of MSCs by generating advanced glycation products (AGEs), oxidative stress and inflammation, which can suppress proliferation, induce apoptosis and increase the production of intracellular reactive oxygen species (ROS). Increased apoptosis and ROS accumulation may be partially responsible for the reduced differentiation potential observed in MS cells [2].

It is important to keep in mind that if a patient with metabolic syndrome criteria is to be treated, the results of a regenerative treatment may fall short of its expectations. The definition of the criteria may vary between different medical societies, but the syndrome is usually identified in the presence of 3 of the 5 criteria presented in Table 40.1 [1].

TABLE 40.1 Diagnostic Criteria for Metabolic Syndrome

Criterion	Definition
Abdominal Obesity	Waist circumference: men > 40 in. (>102 cm); women > 35 in. (>88 cm)
Hypertriglyceridemia	>= 150 mg/dL
Low HDL-C	Men < 40 mg/dL; Women < 50 mg/dL
High Blood Pressure	>= 130/85 mmHg
High Fasting Glucose	>= 110mg/dL

In the next topics some therapeutic strategies, that are used to minimize the deleterious effects of the metabolic syndrome on the health of patients, will be discussed. In this way, it is possible to achieve better results in regenerative therapies and also improve the quality of life of the patients.

SLEEP QUALITY

Experimental studies on the effects of acute sleep deprivation in humans have shown that mediators of inflammation are altered by sleep loss. Such shifts in basal inflammatory cytokines are known to be associated with future development of MS in healthy, asymptomatic individuals [4].

Ample evidence suggests that sleep and pain are related. Recent experimental studies suggest that sleep disturbance may impair key processes that contribute to the development and maintenance of chronic pain, including endogenous pain inhibition and joint pain. It is also important to quote that sleep and the circadian system exert a strong regulatory influence on immune functions [5].

For the reasons mentioned above, it must be considered the importance of achieving sleep quality in the candidates for regenerative therapy. It should be attempted not only to increase the duration of sleep

for a period above 7 hours, but also to improve the quality of sleep, characterized by the alternation between REM and non-REM sleep cycles [3].

COMPLEMENTARY EXAMS

A very important aspect in the pre-treatment evaluation is the complementary exams. It will discuss some of the tests, that are considered extremely important for giving the physician a good characterization of patient's metabolic state.

1. Blood Count

This is a simple and inexpensive test capable of providing important data, such as the level of hemoglobin, leukocytes and platelets. A low level of hemoglobin directly affects the body's regenerative capacity, as it is essential for transporting oxygen to areas, where there is increased inflammatory activity, often suffering from a local state of tissue hypoxia. Leukocytes and platelets are important cellular components for different forms of regenerative treatment, such as platelet-rich plasma and bone marrow aspirate [6]. Therefore, its quantification becomes important for a good choice of therapeutic modality.

2. C Reactive Protein

C-reactive protein (CRP) is an acute inflammatory protein that increases up to 1,000-fold at sites of infection or inflammation. CRP is synthesized primarily in liver hepatocytes but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes [6]. Ultrasensitive C reactive protein should be used as an important marker of inflammation in the body, and a decrease in its plasma levels reflects a positive result in regenerative treatment.

3. Ferritin

The iron storage protein ferritin is also a well-known inflammatory marker. It correlates with biomarkers of cell damage, with biomarkers of hydroxyl radical formation (and oxidative stress) and with the presence and/or severity of numerous diseases.

It is important to know that 95% of patients with high levels of ferritin in their blood have increased inflammatory activity in the body [7]. Only 5% of these individuals have high levels of ferritin due to large amounts of iron in the body. To differentiate these two groups, the measurement of transferrin saturation (protein responsible for transporting iron in the blood) are used. With transferrin saturation at levels below 45% in a patient with high levels of ferritin, the systemic inflammatory state is confirmed.

4. Homocysteine

Elevated homocysteine levels cause osteoblast dysfunction via mitochondrial oxidative damage. This leads to a higher occurrence of fractures and a higher risk of osteoporosis. High levels of homocysteine negatively affects wound healing and is also considered an important inflammation marker [8]. Homocysteine inhibits the synthesis of insoluble collagen fibrils by interfering with normal cross-linking. From the perspective of cartilage homeostasis, these changes in matrix organization interfere

with chondrocyte-mediated mineralization potentially altering the function and properties of calcified cartilage. The transformation of homocysteine into methionine requires some cofactors such as vitamin B12, B6 and B9 [9]. In that way, replacing these vitamins seems to be quite reasonable.

5. TSH, Free T3 and Free T4

The analysis of thyroid function should also be investigated for regenerative therapy. Hypothyroidism can cause the healing process to slow down and may directly affect the outcomes [10]. Therefore, achieving a hormonal balance becomes essential.

Many studies demonstrate the beneficial effects of thyroid hormones on increasing the biochemical content of cells, more specifically, enhancing the collagen production in cultured chondrocytes [11]. Other studies are being conducted to evaluate the potential of thyroid hormones to enhance the functional properties of articular chondrocytes, which remains somewhat understudied.

6. Testosterone

The anabolic effect of testosterone on bone and cartilage is well known. This effect, however, is not unique result of a single action of testosterone on the tissues. Testosterone does stimulate mRNM expression of osteoprotegerin and thereby inhibits osteoclastogenesis much like DHEA and TGF-beta do. There appears to be a combined effect on bone by testosterone, IGF-1 and estradiol [12].

Testosterone is also very important for the maintenance or recovery of the muscle mass, something that has a direct influence on the outcome of various therapies. Low testosterone levels, in both men and women, favor an increase in muscle catabolism and increased levels of body fat. Testosterone stimulation increases the proliferation and preserves stemness of mesenchymal stem cells and endothelial progenitor cells suggesting that, besides other factors, the hormone may engineer these cells and increase their therapeutic potential.

7. DHEA

Dehydroepiandrosterone (DHEA) is a 19-carbon steroid hormone that is classified as an adrenal androgen. DHEA has been shown to antagonize catabolic mediators of cartilage and may exert protective effects in OA, including suppressing matrix metalloproteinases (MMPs) and inducing cartilage restoration [13]. The author's recent research showed that DHEA demonstrated beneficial effects on OA by influencing the balance between the aggrecanases and tissue inhibitors of metalloproteinase-3 (TIMP-3) in cartilage tissues, suggesting that DHEA might protect articular cartilage from degeneration at the molecular level.

8. Osteocalcin

As osteocalcin is produced by osteoblasts, it is often used as a marker for the bone formation process. It has been observed that higher serum osteocalcin levels are relatively well correlated with increases in bone mineral density during treatment with anabolic bone formation drugs for osteoporosis, such as Teriparatide. In many studies, osteocalcin is used as a preliminary biomarker on the effectiveness of a given drug on bone formation [14].

In its carboxylated form (vitamin K2 dependent) it binds calcium directly and thus concentrates in bone, but recent evidence has revealed that it does play an important role beyond bone mineralization. In its uncarboxylated form, osteocalcin acts as a hormone in the body, signalling in the pancreas, fat, muscle, testes, and brain to improve metabolic state [15].

9. Serum Protein Electrophoresis

A serum protein electrophoresis is a simple method that allows proteins to be separated from human plasma into fractions. Its interpretation brings useful information to the regenerative doctor. Thus, it is important for the investigation and diagnosis of several diseases by dosage of albumin, alpha-1-globulin, alpha-2-globulin, beta-globulin and gamma globulin. Albumin is a general health biomarker and its loss is associated with poor healing capacity. Alfa-globulin fractions have increased levels in inflammatory, infectious and immune processes [16]. The increase in beta-globulin is observed in situations of disturbance of lipid metabolism or iron deficiency anemia. The decrease or absence in the gamma fraction indicates congenital or acquired immunodeficiencies. Its increase suggests a polyclonal increase in immunoglobulins associated with inflammatory, neoplastic (multiple myeloma and lymphoproliferative disorders) or infectious conditions [17]. The knowledge of these patterns helps the physician to assemble the general patient status.

10. Alkaline Phosphatase

Most of the alkaline phosphatase (ALP) isoenzymes are derived from the bones and liver. High levels of ALP are often encountered during routine blood investigation in elderly patients. Osteoporosis may increase its blood levels up to 3-5 times normal. Bone pathology causes of elevated alkaline phosphatase include Paget's disease, hyperparathyroidism, osteomalacia, metastatic bone disease and a recent fracture [18]. By these reasons, it is imperative to investigate bone turnover specially when considering to use MSCs for regenerative purposes.

NUTRACEUTICAL OPTIONS FOR CANDIDATE PATIENTS FOR REGENERATIVE INTERVENTION

1. Ômega-3

It has been a long time since obesity is considered one of the main causes to mechanical stress, cartilage degradation and OA [19]. However a new paradigm has shifted the mechanical idea to the body inflammation caused by the adipose tissue leading to bone, tendon and cartilage degeneration through the secretion of adipokines. In these context, omega-3 polyunsaturated fatty (N-3 PUFAs) acids has been shown to reduce the expression of inflammatory markers, cartilage degradation and oxidative stress in chondrocytes [20]. On the opposite direction, these markers were increased on omega-6 polyunsaturated fatty (n-6 PUFAs) acid and saturated fatty acid stimulation. There is also evidence indicating that chronic "bad" fatty acids intake can trigger inflammation through toll-like receptor (TLR)-4 and TNF-a in human tissue [21]. The pro-inflammatory and pro-apoptotic actions of saturated fatty acids and N-6 PUFAs were partially reduced on N-3 PUFAs stimulation by means of prostaglandin reduction in human chondrocytes [22].

Effects of N-3 PUFAs has also been shown on pain and function improvements. Gruenwald et al. investigated in a randomized, double blind controlled trial the effects of adding n-3 PUFAs to glucosamine sulphate therapy in hip and knee OA patients. They found a higher number of patients with reported WOMAC pain reduction and range of motion improvements compared to mono-therapy with glucosamine sulphate [23]. In summary, multiple animal and intervention studies have shown a beneficial effect of a diet high in n-3 PUFAs or with a low n-6:n-3 PUFA ratio, with a decrease in cartilage degradation and less osteophyte formation [24].

2. Magnesium

Magnesium is the second most abundant intracellular cation, and is a critical cofactor for any reaction mediated by ATP consumption, controlling more than 300 enzimatic reactions [25]. By acting as a calcium channel antagonist, it plays an important role in activities regulated by intracellular calcium concentration fluxes, such as muscle contraction and insulin release (glucose metabolism).Low magnesium intake has been associated with elevated serum levels of C-reactive protein (CRP) and TNF-a, especially in subjects with metabolic syndrome [26]. Conversely, oral magnesium supplementation decreased CRP levels [27]. In another study, the serum Mg concentration was inversely associated with the prevalence of metabolic syndrome, diabetes mellitus and hyperuricaemia in patients with radiographic knee OA [25].

It has also been shown that low magnesium intake and hypomagnesemia is associated with worse pain and function scores in knee OA subjects [28]. Additionally, magnesium likely plays a role as a pain mediator, and has been shown to alter the levels of inflammatory cytokines and neurotransmitters in human and animal models [29]. Increasing evidence in recent years has been describing that magnesium deficiency is active in several pathways that have been implicated in OA, including increased inflammatory mediators (that ultimately result in NF-kB activation), cartilage damage, defective chondrocyte biosynthesis, aberrant calcification and a weakened effect of analgesics [30]. Furthermore, the nutritional supplementation of Mg^{++} or local infiltration may represent a prophylactic treatment and may slow the OA progression [31].

3. Coenzyme Q10

Coenzyme Q10 (CoQ10) or ubiquinone is a lipid-soluble vitamin-like antioxidant naturally found in the diet and can also be synthesized endogenously by all cells of our body. It is one of the key components in ATP production in electron transport chain in mitochondria. CoQ10 protects membranes against oxidation, regenerates and reduces vitamins E and C and enzymatic antioxidant systems, and modulates prostaglandin metabolism [32]. In a recent randomized controlled trial, 100mg/day of CoQ10 supplementation showed beneficial effects on inflammatory cytokines (TNFa, IL-6) and oxidative stress (malondialdehyde – MDA) in rheumatoid arthritis patients [33]. Another randomized controlled trials assessed CoQ10 effects on a 500mg/day supplementation for at least 12 weeks with similar findings [34]. Finally, a systematic review provided some evidence that CoQ10 supplementation may improve the process of inflammatory state in patients with metabolic diseases [35].

4. Vitamin D

Increasing evidences from laboratory studies have shown the positive effects of vitamin D (1a,25$(OH)_2D_3$) on articular cartilage. Vitamin D receptors are expressed in human articular chondrocytes of OA cartilage, particularly in the superficial zone. Vitamin D has shown to remarkably enhance bone-marrow stem cells (BMSCs) viability, migration and chondrogenic differentiation. These alterations of BMSCs induced by vitamin D were reinfored by TGF-β1 overexpression (a potent growth factor for chondrogenesis and cartilage health) while were reversed by TGF-β1 silencing [36].

Several studies has been relating the relationship of vitamin D and cartilage status with TGF-b1 expression in articular cartilage [37, 38]. Results of a extensive cartilage degeneration model study showed that 1a,25$(OH)_2D_3$ supplementation enhanced the TGF-b1 expression in the articular cartilage of ovariectomized rats (OVX), and vitamin D deficiency (VDD) suppressed it [39]. The expression of MMP-9,-13 in articular cartilage increased with OVX and VDD, and decreased with 1a,25$(OH)_2D_3$ supplementation. Vitamin D significantly counteracted the increased C-telopeptide of type II collagen (CTX-II), an important biomarker of cartilage matrix degeneration, release due to TFNa stimulation.

A new effect vitamin D in synovial fibroblast (FLS) invasion in arthritis has been described, suggesting that the reduced serum levels of vitamin D and its metabolites commonly seen in rheumatoid patients might increase risk for FLS-mediated cartilage and bone invasion and erosions [40]. Treatment with vitamin D has the potential to become a helpful adjuvant aimed at reducing cartilage and bone destruction. Recent analysis suggests that mean serum 25(OH)D levels of about 75 to 110nmol/l provided optimal benefits without increasing health risks in humans [41].

5. Vitamin B12

During the last years, vitamin B12 has gained particular attention since epidemiological studies have reported a correlation between B12 status and bone quality and fracture risk [42]. The micronutrient B_{12} is involved as a cofactor in two enzymatic reactions. The enzyme methionine synthase requires B_{12} for the remethylation of homocysteine into methionine. The other enzyme dependent on B_{12} is methylmalonyl-CoA mutase, which catalyzes the conversion of methylmalonyl-CoA into succinyl-CoA. As well kwown, homocysteine is a highly inflammatory marker for several diseases, including osteoporosis. Recent study has found that homocysteine and methylmalonic acid induced osteoclastogenesis [43].

6. Vitamin K2

Vitamin K is a fat-soluble vitamin family present in many foods including vegetables, fish, meat, cheese and eggs. Vitamin K2 (MK-7) is more effective in catalysing osteocalcin carboxylation in bone and counteracting coumarin anticoagulants in the liver than vitamin K1 [44]. A study showed that vitamin K2 (VK2) influences skeletal muscle cells (satellite cells) with enhanced proliferation, differentiation and cell migration *in vitro*, demonstrating the potential of vitamin K2 in maintaining normal muscle function from quiescent cells differentiation [45].

There is also credible evidence to support the effects of vitamin k2 on differentiation of mesenchymal stem cells into osteoblast. Runx2 is an early osteogenic marker that regulates BMSC shape and induces

osteoblast differentiation. VK2 has shown to promote transcription of osteocalcin (OCN) protein during osteogenic differentiation [46]. Vitamin K2 also inhibit osteoclast formation indirectly by decreasing the expression of RANKL and increasing the expression of osteoprotegerin (osteoclast inhibitory factor) in human stromal cells. Vitamin K2, in this way, is capable to drive MSCs differentiation towards osteogenic instead of adipogenic lineages [47].

7. Curcumin

There has been increasing attention on the use of curcumin for regenerative medicine applications. Most studies on curcumin have focused on the wound healing process. However, there has been a gradually broadening of applications to reconstruct other tissues like tendon and bone [48]. Curcumin has been shown to be an effective scavenger of reactive oxygen species (ROS) and to exert cytoprotective effects against H_2O_2-induced oxidative stress in cultured retinal pigment epithelium (RPE) cells. Curcumin exerts an antioxidant effect by affecting reactive species; scavenging superoxide anion (O–), peroxynitrite (NOO), nitric oxide (NO), peroxyl radicals (ROO), and hydroxyl (OH–) radicals and inducing an up-regulation of antioxidant proteins. Curcumin has been reported to reverse liver, brain and kidney damage, hyperglycemia, lipid oxidation, inflammation, and oxidative stress [49].

Several mechanisms by which curcumin can exert anti-inflammatory activity have been suggested. It was proposed that curcumin relieves oxidative stress and inflammation in chronic diseases by its effects on the Nrf2-keap1 signaling pathway [50]. Moreover, curcumin can suppress pro-inflammatory pathways associated with most chronic conditions and block both the production of tumor necrosis factor (TNF) and the cell signaling mediated through TNF in different cell types [51]. Because of the chemical structure of curcumin, it may act as a natural-free radical scavenger which can reduce the release of various interleukins over NF-κB [52]. Curcumin may mimic the stress response that induces some components of the protein homeostasis network. Authors routinely evaluate the use of Long Turmeric 400mg + Piperine 4mg twice a day, with meals, for patients in regenerative treatment in advance.

8. Resveratrol

Resveratrol (RSV) exhibits reliable and extensive regenerative activities that have received increasing clinical attention. In cultures of MSCs, RSV promoted cell aggregate formation and improved their osteogenic potential, with anti-TNFa effects in the proliferation capacity of these cells. Several mechanisms of the effects of RSV on the survival, self-renewal, and lineage commitment of MSCs in vitro and in vivo have also been described [53].

Resveratrol has been shown to protect MSCs from senescence and aging. Moreover, RSV can activate Sirtuin 1, a longevity gene related to many diseases associated with aging [54]. Sirtuin 1 can regulate the expression of extracellular matrix (ECM)-related proteins, promote mesenchymal stem cell differentiation, play anti-catabolic, anti-inflammatory, anti-oxidative stress, and anti-apoptosis roles, participate in the autophagic process, and regulate bone homeostasis in OA [55].

9. Probiotics

There is some evidence showing that the linking factor between metabolic abnormalities and the OA onset could be represented by the persistence of a chronic low-grade systemic inflammation [56]. These findings support the idea of a new phenotype of OA, a metabolic OA, in addition to the age-related and injury-related phenotypes. In this regard, many inflammatory pathways that lead to bone, cartilage and soft tissues degeneration may have been downregulated with a health gut microbiome [57].

It has been shown that in genetically susceptible individuals, environmental factors (e.g., diet, smoke and alcohol) can disturb the gut microbial populations, causing dysregulations in host innate and adaptive immune systems, leading to the development of several diseases [57]. Manipulation of the gut microbiome presents valuable avenues for therapeutic and clinical applications, including musculoskeletal diseases. Currently, well-known gram-positive bacteria that confer such health benefits include Bifidobacterium and Lactobacillus [58]. Thus it seems reasonable to orient any candidate patient of regenerative treatment to enhance the gut health in order to boost the effects of a such therapy.

CONCLUSION

All attempt should be made to increase the potential benefits of any given regenerative treatment. The measures exposed above are ones that can be easily implemented in point of care and should be considered a "preparing the soil" before cells or plasma derivatives are seeded.