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BONE TURNOVER AND FRACTURE RISK IN POSTMENOPAUSAL WOMEN WITH TYPE 2 DIABETES

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Tesi di Dottorato della

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Abstract

Introduction. Bone fragility is increasingly recognized as a complication of type 2 diabetes (T2DM). However, the underestimation of fracture (Fx) risk in T2DM using the classical assessment tools pointed out the need of clinical recommendations for the evaluation of bone health in T2DM. The multidisciplinary expert panel chaired by Chiodini suggested the diagnostic approaches for the detection of T2DM patients worthy of bone-active treatment (Chiodini I et al, Nutr Metab Cardiovasc Dis. 2021).

Aims. The aim of the study was to apply these algorithms to a well characterized cohort of postmenopausal women with T2DM to validate them in clinical practice.

Materials and methods. The presence of the major T2DM-specific Fx risk factors (a disease duration ≥10 years, one or more chronic T2DM complications, the use of insulin or thiazolidinediones and persistent poor glycaemic control) was ascertained at baseline in 107 female subjects with T2DM. They were conservatively followed-up. At baseline and after follow-up we evaluated bone mineral density (BMD) and the presence of clinical and morphometric vertebral Fx.

Results. Following the flow-charts, 34 (31.8%) and 73 (68.2%) patients would have been pharmacologically and conservatively treated, respectively. Among the 49 patients without both clinical Fx and major T2DM-related risk factors, who would have been, therefore, conservatively followed-up, only one subject showed a prevalent vertebral Fx (sensitivity 90%, negative predictive value 98%). After the follow-up the BMD variation in T2DM patients who would have been pharmacologically treated did not differ from that observed in T2DM patients who would have been conservatively followed-up. Two patients experienced an incident Fx and both would have been pharmacologically treated at baseline.

Conclusions. The clinical consensus recommendations established by the Italian multidisciplinary expert panel performed well in our sample of postmenopausal women with T2DM. Indeed, among those subjects with bone-active treatment indication as many as 15.3% of patients experienced an incident Fx fracture (incident rate 30 Fx per 1000 patient-years), thus confirming a high Fx risk worthy of specific treatment and, conversely, among those subjects without bone-active treatment indication no incident Fx were observed.

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I. Introduction

1. Type 2 diabetes bone fragility

Type 2 diabetes (T2DM) and osteoporosis are both highly prevalent chronic disorders associated with severe morbidity and increased mortality. Individuals with T2DM have an increased risk of bone fragility fractures compared to non-diabetic subjects. Accordingly, nowadays skeletal fragility is considered a T2DM-related complication [1]. However, the true prevalence of this complication can be hard to be determined, as the increased fracture risk in patients with T2DM, can be anyway underestimated by conventional WHO criteria for osteoporosis [2]. Indeed, in T2DM patients for any given T-score of bone mineral density (BMD) the fracture risk is increased with respect to the general population and fragility fractures may occur despite a normal or even augmented BMD, thus suggesting a deterioration of bone quality rather than of bone quantity in the pathogenesis of T2DM-related bone fragility.

1.1. Pathophysiology

Several pathophysiological mechanisms have been implicated in the altered bone quality of T2DM [3].

From a cellular and molecular point of view, accumulation of advanced glycation end-products with non-enzymatic glycosylation of collagen, decreased bone turnover, the presence of a pro-inflammatory state, oxidative stress and, eventually, microvascular disease and the increase in bone marrow adiposity determine micro- and macro- architecture abnormalities causing reduced resistance to mechanical stress.

From a clinical point of view, the T2DM duration, the poor glycaemic control and the presence of T2DM-related complications (poor balance, visual impairment, peripheral neuropathy, impaired renal function) have been associated with an increased risk of falls and fractures.

Finally, certain medications for T2DM treatment negatively affect skeletal health: the use of thiazolidinediones, sodium-glucose cotransporter-2 (SGLT2) inhibitors and insulin (this latter not

directly but probably through an increased risk of hypoglycaemic events) can contribute to the increased fracture risk in T2DM patients [3].

1.2. Assessment

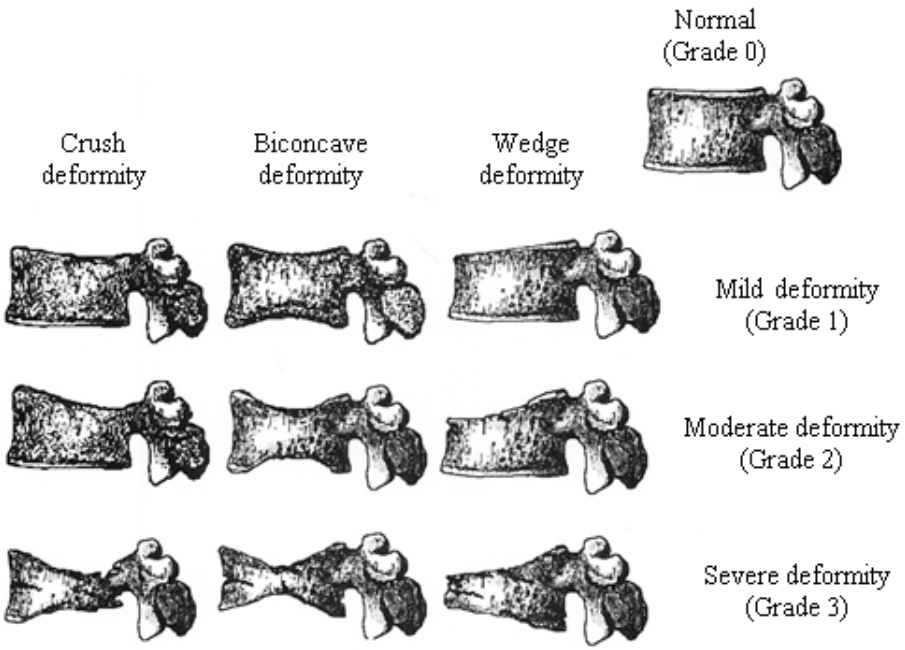
Dual X-Ray Absorptiometry (DXA). DXA is the conventional technique for the diagnosis of osteoporosis according to WHO criteria. However, although BMD measured by means of DXA remains a significant independent predictor of fracture risk also in T2DM, for a given BMD T-score and age fracture risk in T2DM patients is higher than in the general population, so that in diabetics fractures may occur despite a normal or even increased BMD [2, 4]. For each 1 SD decrease in BMD, the risk of hip fracture is almost equally doubled in both individuals with and without T2DM, but it has been estimated that diabetic subjects have a risk of hip fracture similar to non-diabetic subjects in the presence of 0.6 SD and 0.4 SD higher BMD levels in women and men, respectively [4]. Therefore, the evaluation of BMD alone cannot represent a reliable tool for the estimation of fracture risk in T2DM. Likewise, algorithms such as the WHO Fracture Risk Assessment Tool (FRAX) underestimate the fracture risk in T2DM. Other abnormalities in bone microarchitecture and/or in the intrinsic properties of bone itself not captured by DXA are likely responsible for this augmented fracture risk in T2DM [5].

Vertebral fractures assessment. A long T2DM duration (i.e., >10 years), insulin treatment and the presence of the T2DM-related chronic complications are associated with fragility fractures, regardless of BMD [5]. Therefore, in these situations as well as in patients with previous clinical fragility fractures, the vertebral fracture assessment should be mandatory in addition to BMD measurement by DXA [6], particularly in the presence of a poorly controlled disease. Indeed, up to a third of postmenopausal T2DM women investigated by a lateral spinal radiograph showed asymptomatic morphometric vertebral fractures [7], that *per se* represent a major risk factor for subsequent fractures [8].

Conventional spinal X-rays represents the most used technique for the detection of vertebral fractures. However, the addition of lateral spine imaging technology to DXA for vertebral fracture assessment (VFA), represents an alternative tool for this purpose [9].

Any reduction in anterior, middle, and posterior heights higher than 20% represents a vertebral deformity, which often, but not always, identifies a vertebral fracture. Several methods can be utilized which rely on either visual assessment for vertebral deformity, morphometric measurement of the change in vertebral height, or some combination of both. The most used method and that recommended by the International Society of Clinical Densitometry (ISCD) with VFA is the semiquantitative visual method of Genant. According to this method, vertebrae from T4 to L4 are graded on visual inspection and without direct vertebral measurement as normal (grade 0), mildly deformed (grade 1, approximately 20-25% reduction in any height), moderately deformed (grade 2, approximately 25-40% reduction) and severely deformed (grade 3, approximately more than 40% reduction [10]. (Figure 1). The increasing severity of vertebral fractures was associated with progressively worse bone microarchitecture [11]. Accordingly, the Spinal Deformity Index (SDI), which is the sum of the vertebral fracture grades across each of the vertebrae in the spine from T4 to L4, represents an accurate tool for predicting future vertebral fracture risk [12].

Figure 1. The classification of vertebral fractures according to the semiquantitative visual method of Genant (adapted from Genant et al. [10])



Other imaging modalities, including MRI and CT, may also be used to identify vertebral fractures, but higher costs, lower availability and, for CT, higher radiation exposure limit their use in clinical practice.

Other imaging techniques. Additional tools that can be applied to DXA have been investigated in T2DM. Several studies found a reduction of Trabecular Bone Score (TBS), a textural index based on the evaluation of pixel grey-level variations in the lumbar DXA image, providing an indirect index of bone architecture, in T2DM. In the diabetic population TBS could predict fracture risk better than BMD [5]. In some cohorts of T2DM patients, the hip structural analysis (HSA), that give information on bone geometry and indirectly on bone resistance to axial compressive forces, showed a weaker geometry and a compromised skeletal load response [6]. However, the additive role of HSA on the prediction of fracture risk in T2DM remains to be established.

By use of quantitative ultrasound (QUS), QUS parameters were found to be significantly decreased in patients with T2DM compared with controls, but their ability to discriminate T2DM patients with and without fragility fractures is probably limited [13].

Recently, peripheral quantitative computed tomography (QCT) and high-resolution peripheral QCT of the distal radius and tibia have been employed in T2DM, but the results have been quite inconsistent. Several studies, although not all, suggest in T2DM preserved indexes of trabecular microarchitecture associated but increased cortical porosity with a consequent deficit in biomechanical properties, particularly in diabetic females with fragility fractures [6].

Finally, the use of magnetic resonance imaging at both peripheral and axial skeleton for the assessment of trabecular and cortical bone parameters and of the composition of bone marrow fat (found altered in T2DM postmenopausal women with fragility fractures) could help in the future in the fracture risk estimation in T2DM [6].

However, despite some promising results, the clinical relevance of imaging techniques other than DXA and X-rays for the prediction of fracture risk in T2DM patients need to be confirmed on a prospective basis and their scarce availability and high costs do not consent their routine use.

Bone turnover. In T2DM patients, histomorphometric studies have shown a reduction of the osteoblast number and of the osteoid amount and a low bone formation rate. In addition to a decrease in the activation frequency of the bone, in T2DM the degree of bone mineralization and the non-enzymatic collagen crosslinking by pentosidine, which has been proposed as a bone fragility marker in T2DM, were found to be increased and directly associated with glycated haemoglobin (HbA1c) levels [5], thus consistent with a relatively low bone turnover state.

The low bone turnover state in T2DM has been confirmed even in clinical studies. In particular, both bone formation, as mirrored by osteocalcin (OC) levels, and bone resorption, as evaluated by C-terminal telopeptide of type I collagen (CTX) levels, were found to be reduced and negatively associated with metabolic control [14]. Consistent with a low bone turnover in T2DM, other markers of bone apposition and resorption, such as the procollagen type 1 amino-terminal propeptide and the N-terminal telopeptide of type I collagen, respectively, were found to be reduced in patients with T2DM than in non-diabetic controls [15]. At variance, alkaline phosphatase total activity has been found to be increased in T2DM patients than in non-diabetic individuals [14]. Notwithstanding the potential role of low bone turnover in impairing the bone quality in T2DM, the use of bone turnover markers in the prediction of fracture risk in T2DM is still a matter of debate.

Furthermore, a few studies have suggested that a condition of relative hypoparathyroidism, eventually caused by a calcium-sensing defect or secondary to chronic hypomagnesaemia, could contribute to low bone turnover in diabetic patients. Finally, osmotic diuresis induced by glucosuria causes renal calcium leakage that can lead to a negative calcium balance. In keeping with this, improvement of glycaemic control is associated with a reduction in urinary levels of calcium in T2DM [5].

1.3. Management

The use of classical fracture risk assessment tools underestimates the risk of osteoporotic fractures in T2DM. Indeed, for a given T-score and age or for a given FRAX (the WHO Fracture Risk Assessment algorithm) score, diabetic individuals have a higher fracture risk than controls. To date, surrogate measures have been proposed for a more accurate estimation of fracture risk in T2DM using

the FRAX tool, such as adding the lumbar spine TBS, using rheumatoid arthritis as a proxy for T2DM or decreasing the actual BMD hip T-score of the diabetic patient by 0.5 units [16]. However, notwithstanding each of the proposed methods was able to improve FRAX performance, no single method was found to be optimal in all settings of T2DM and fracture risk remained underestimated by these approaches in this kind of patients, particularly in those with long-lasting disease [17].

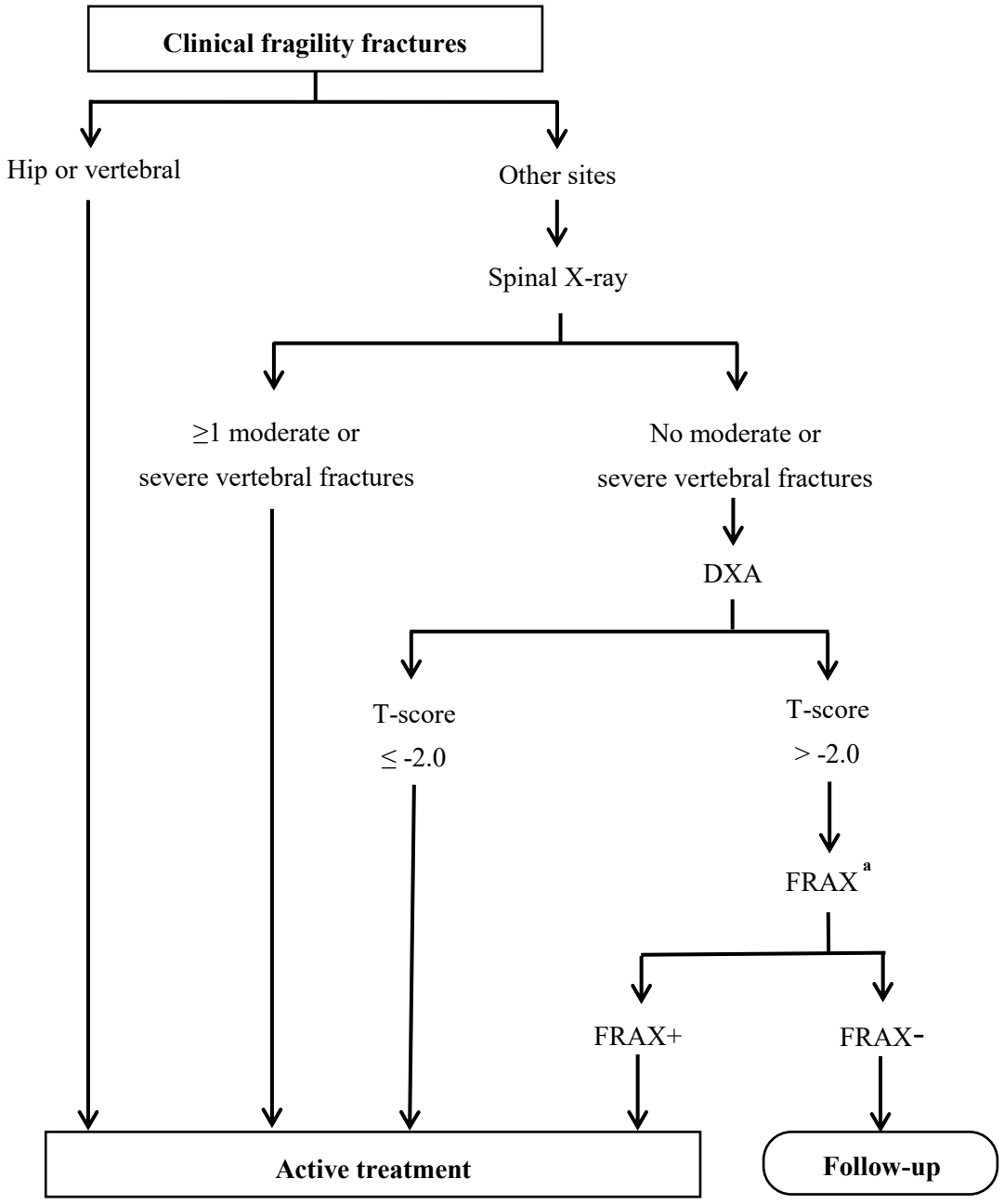
An Italian interdisciplinary task force of experts suggested the use of the following major T2DM-specific risk factors for fracture for the stratification of fracture risk in T2D patients: a disease duration above 10 years, the presence of one or more chronic T2DM complications, the use of insulin or thiazolidinediones and persistent poor glycaemic control (i.e. HbA1c levels above 64 mmol/mol for at least 1 year) [18]. The same experts panel defined that the fracture risk in T2DM patients should firstly rely on the presence or not of a previous fragility fracture and on the individual risk profile, with the inclusion of the above T2DM-specific risk factors [18]. Accordingly, two independent diagnostic approaches were suggested in the presence or the absence of a prevalent fragility fracture, respectively, as depicted in Figure 2. The two flowcharts allow to identify the different clinical situations for which the use of bone-active drugs is suggested [18], in addition to lifestyle intervention with medical nutrition therapy and exercise which form the cornerstone of therapy of T2DM and coexistent osteoporosis [1]. Indeed, adequate calcium and vitamin D intakes, avoidance of smoking and of excessive alcohol consumption, weight-bearing exercises and prevention of falls should be recommended also in T2DM patients.

With regard to anti-osteoporotic medications, it is generally accepted that patients with T2DM should receive the same bone-active drugs as non-diabetic patients [1, 18]. However, apart from teriparatide that has been tested in T2DM of both genders, data on the efficacy of these drugs on fracture risk reduction in T2DM are limited and mainly based on studies of postmenopausal T2DM women, generally with low baseline BMD levels, this representing a limited subset of T2DM patients at increased fracture risk [18].

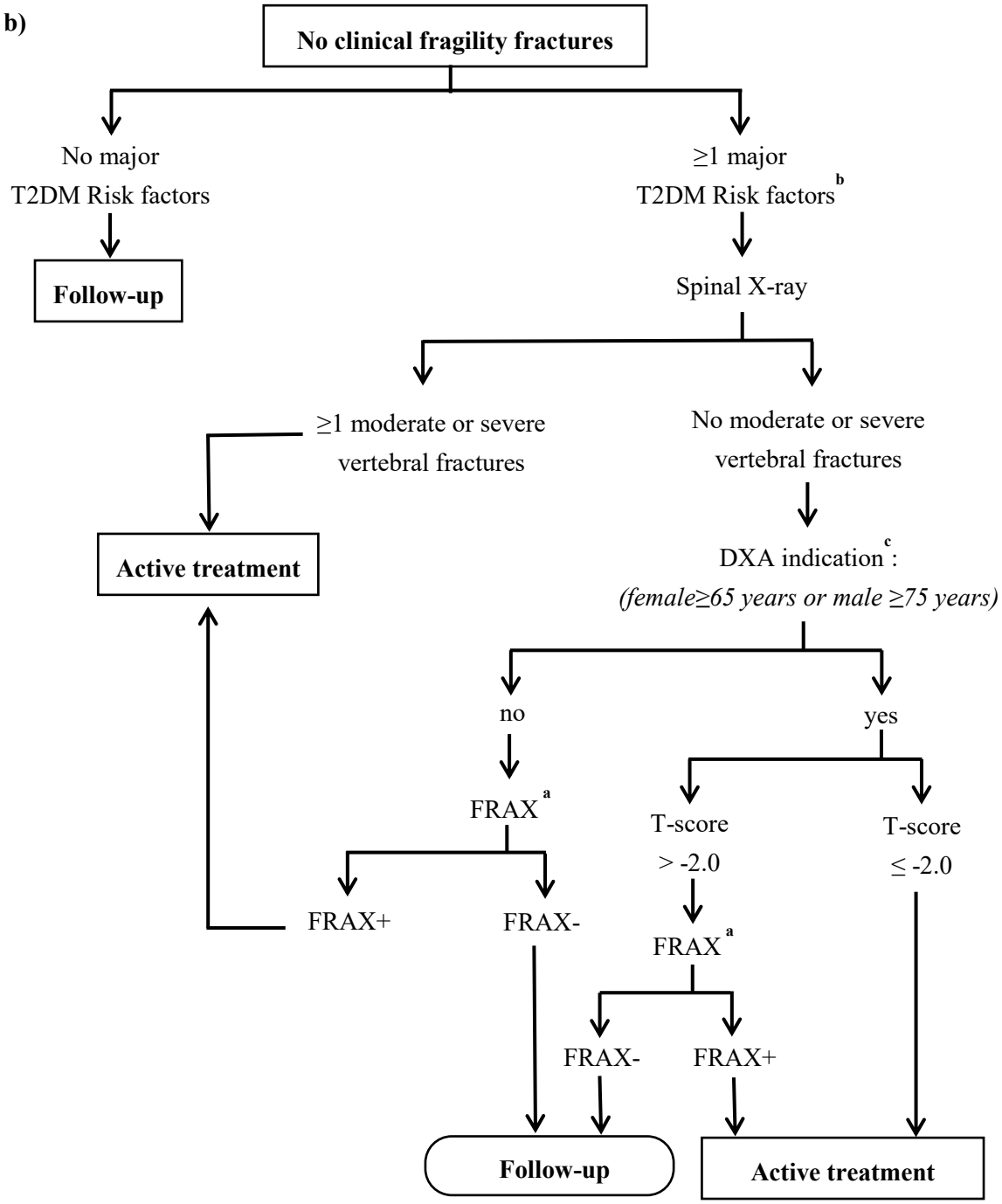
Further clinical trials in diabetic patients are then needed to determine the efficacy and safety of available anti-osteoporotic agents in this kind of patients.

Figure 2. The diagnostic approach suggested by the expert panel in the presence (a) or in the absence (b) of a prevalent clinical fragility fracture (modified from Chiodini et al. [18])

a)



b)



DXA: Dual X-Ray Absorptiometry. FRAX: WHO Fracture Risk Assessment algorithm. T2DM: type 2 diabetes. [a]FRAX criteria to be calculated without bone mineral density and with rheumatoid arthritis as surrogate risk factor of diabetes (FRAX+, patients who fulfill the National Osteoporosis Foundation criteria for treatment: 20% ten years risk of major fragility fractures and 3% ten years risk of hip fracture); [b]major T2DM risk factors for fracture: 1) diabetes duration >10 years, 2) insulin and/or thiazolidinedione treatment, 3) chronic diabetes complications, 4) glycosylated haemoglobin levels above 64 mmol/mol for at least 1 year; [c]DXA analysis to be performed in females ≥65 years or males ≥75 years, according to the International Society of Clinical Densitometry indications for DXA [19].

2. Gut-bone axis

2.1. Incretin hormones

GLP-1 and GLP-2. Glucagon-like peptides 1 and 2 (GLP-1 and GLP-2) are gut-derived peptides secreted from specialized entero-endocrine 'L' cells located predominantly in the ileum and colon, in response to nutrient ingestion [20]. They share with glucagon a common precursor molecule, proglucagon. Proglucagon is stored in intracellular granules where it undergoes posttranslational processing and is cleaved by prehormone convertase 1/3 (PC1/3), to yield glucagon-like peptides [21].

GLP-1 and GLP-2 exhibit a wide range of functions. GLP-1 has primarily insulinotropic and glucagonostatic properties. Nutrient ingestion, especially of carbohydrates and fat, is the strongest stimulant for its secretion. GLP-1 induces insulin secretion in pancreatic beta cells, stimulates somatostatin release and suppress glucagon secretion in a glucose-dependent manner [20]. Moreover GLP-1 influences the function of multiple organ systems: chief among these are the inhibition of gastrointestinal motility and acid secretion and the central effect on appetite regulation. Finally, it likely has cardio-protective and neuro-protective effects. GLP-2, on the other hand, increase glucagon levels and has intestinotropic properties.

GLP-1 and GLP-2 are susceptible to degradation by a serine protease, dipeptidyl peptidase-IV (DPP-IV) [22]. This enzyme is widely distributed. GLP-1 degradation by DPP-IV is really rapid, such that the half-life of intact GLP-1 is 1-2 minutes. GLP-1 may also be susceptible to degradation by neutral endopeptidase 24.11 (NEP 24.11) [23]. As a result, less than 10% of secreted GLP-1 reaches its target organs intact.

Also GLP-2 is cleaved by DPP-IV, although a little slowly than GLP-1, with a half-life of about 7 minutes. The inactive metabolites of both GLP-1 and GLP-2 are then quickly cleared by the kidneys.

GIP. Glucose-dependent insulinotropic polypeptide (GIP) is secreted from the entero-endocrine 'K' cells, predominantly found in the proximal small intestine. The GIP precursor, proGIP, is cleaved by PC1/3 to yield the 42-amino acid bioactive GIP(1–42). Like GLP-1, GIP is stored in granules and is rapidly released upon nutrient stimulation to perform its insulinotropic effects, then is rapidly cleaved by DPP-4 with a half-life of 7 minutes [24].

2.2. Incretin-based therapies

The insulinotropic effects of incretins allowed the development of several incretin-based therapies for T2DM treatment: the GLP-1 receptor agonists and the DPP-4 inhibitors. The research on GIP-based treatment in diabetics is limited due to a desensitization of the GIP system in T2DM patients.

GLP-1 receptor agonists. Whereas GIP effect is lost in T2DM patients, the pancreatic action of GLP-1 is maintained at supraphysiological doses [25]. Consequently, several GLP-1 receptor agonists resistant to DPP-4 and NEP24.11-mediated degradation as well as renal extraction have been developed for T2DM treatment. The peptide exendin-4, isolated from Gila Monster saliva, appeared to be an agonist of the mammalian GLP-1 receptor [26]. The first GLP-1 receptor agonist approved for treatment of T2DM was exenatide, that is the synthetic form of exendin-4[27]. In the following years, several other GLP-1 receptor agonists were marketed. The covalent linking of GLP-1 to various, larger molecules has resulted in GLP-1 receptor agonists with longer half-lives, thus requiring once-weekly administration, like dulaglutide, which is composed of two stable GLP-1 moieites linked to an immunoglobulin fc fragment [28]. In general, the effects of the GLP-1 receptor agonists are similar to those of endogenously GLP-1, including stimulation of insulin release, inhibition of glucagon release and decreasing gastric emptying rates. Moreover, GLP-1 receptor agonists improve β-cell function, reduce insulin resistance and induce weight loss in various degrees, probably through central effects on appetite and food intake [29].

DPP-4 inhibitors. Another incretin-based T2DM treatment is founded on the inhibition of DPP-4 which induces increased endogenous circulating GIP and GLP-1 levels [30]. DPP-4 inhibitors, like sitagliptin and vildagliptin, are oral drugs approved for T2DM treatment.

2.3. Physiology of the gut-bone axis

Emerging evidence points out a role of incretin hormones in bone homeostasis through the enteroendocrine-bone axis. Through this gut-bone axis, the gut-derived hormones affect bone remodelling either directly or indirectly [31].

Bone remodelling follows a circadian pattern with an increase in bone resorption during night and a decrease during the day. This daily rhythm of bone turnover is determined by several factors, including food intake. Hence, it is diminished by fasting and restored by feeding, thus reflecting feeding pattern [32]. In more detail, bone resorption is reduced after a meal, independently of timing, nearby the peaks in gut hormone secretion, whereas during fasting, when gut hormones remain at basal levels, the morning suppression of bone resorption markers is abolished [31]. Moreover, the diet composition influences the pattern of bone resorption markers, independently of sex and menopausal status: ingestion of glucose, proteins and triglycerides induces an immediate reduction in CTX up to 52% from baseline compared to a 20% reduction during continuous fasting [33].

Markers of bone formation are also influenced by feeding, but their postprandial variation is less apparent, suggesting a transient shift toward skeletal deposition in the postprandial period. Thus, incretin hormones secreted after feeding (GIP, GLP-1 and GLP-2) are able to modulate bone turnover in favour of bone formation. These direct effects of gut hormones on bone formation are then potentiated by inducing insulin and amylin release from pancreatic islets. These other hormones promote bone formation and inhibit osteoclast activity, respectively. Finally, also the gastrointestinal peptides of the neuropeptide Y family, primarily peptide YY (PYY) which is co-secreted with glucagon-like peptides from the 'L' cells and mainly act through the central nervous system, may also have direct effects and autocrine action in bone cells [34]

This gut-bone axis has probably evolved to maximise skeletal strength in times of abundant nutrition and preserve calcium homeostasis in times of poor food availability since the skeleton is the largest body reservoir of calcium [32].

2.4. Potential therapeutic implications

The regulator role of gut in bone homeostasis has risen the possibility of a beneficial effect of incretin-based treatments on bone.

Several GIP analogues enzymatically resistant to degradation have been developed, however preclinical data on their use in healthy rodents are scarce [35]. In type 1 diabetic mice, the administration of GIP mimetic resulted in bone turnover similar to non-diabetic mice and reduction in

matrix collagen destruction without any variation in insulin secretion [36], thus suggesting that these effects are independent of insulin release. In healthy humans, recent evidence suggest that exogenous administration of GIP is able to reduce bone resorption, however further studies are needed to demonstrate whether this effect is direct or indirect. Anyway, a definitive proof of a direct and positive link between the action of GIP and bone comes from a single-nucleotide polymorphism (rs1800437), that results in decreased GIP receptor activity and in perimenopausal women determines a skeletal phenotype characterized by low BMD as well as high incidence of non-vertebral fractures [37]. However, as previously stated, the interest in GIP research in the diabetic field is limited, due to the impaired insulinotropic action of GIP in T2DM.

The efficacy of GLP-1 analogues has been firstly investigated in type 2 diabetic animal models, where liraglutide was capable of ameliorating trabecular and cortical microarchitecture [38]. However, in rodents the dose regimen was about 40-times higher than that used in human clinical trials. In ovariectomized osteoporotic rats the administration of exenatide with a dosage similar to that used in humans, was able to improve trabecular bone mass and microarchitecture and to revert the increase of bone resorption observed after ovariectomy [39].

Despite the extensive use of GLP-1 receptor agonists in T2DM treatment and the availability of many molecules belonging to this class, data regarding their safety and efficacy on bone health and fracture risk in humans are scarce and often discordant [35]. Several years ago, based on the data available at that time, two meta-analyses of randomized clinical trials of GLP-1 receptor agonists concluded that in T2DM patients treated with GLP-1 receptor agonists as compared to other anti-diabetics the fracture risk was neither elevated nor reduced [35, 40]. However, a molecule-based analysis underlined a possible reduction of the fracture risk with liraglutide (OR 0.38 [95% CI 0.17–0.87]) and a higher risk with exenatide (OR 2.09 [95% CI 1.03–4.21]) [40], thus suggesting different effects on the basis of the specific GLP-1 receptor agonist. Accordingly, another subsequent meta-analysis of RCT demonstrated a significant reduction in the fracture risk only with liraglutide and lixisenatide compared with placebo and other anti-diabetic drugs. Moreover, these beneficial effects were dependent on the duration of treatment, being visible only for treatment period of more than 52

weeks [41]. Retrospective cohort studies concluded a neutral role of GLP-receptor agonists on the risk of fracture [42, 43]. Finally, the Bayesian network meta-analysis by Zhang and coauthors suggests that GLP-1 receptor agonists were associated with a decreased fracture risk compared to placebo or other anti-hyperglycemic drugs and, among GLP-1 receptor agonists, exenatide was the best option agent with regard to the risk of fracture [44]. However, a controlled trial designed to assess the fracture risk on treatment with GLP-1 receptor agonists treatment with bone fracture as the primary outcome is still lacking.

Similarly, as regards the inhibition of DPP-4, data from some clinical trials suggest a potential favourable effect of DDP-4 inhibitors on bone metabolism, but a clear evidence is still missing. A meta-analysis of 28 clinical trials including over 21,000 patients with T2DM on various agents of this class showed that treatment with DPP-4 inhibitors was associated with a reduced fracture risk compared with placebo or other anti-diabetic treatments [45].

Whereas other subsequent studies on a single molecule of the class (saxagliptin and sitagliptin respectively) found no effect on fracture risk [46]. In a retrospective population-based study from the UK, fracture risk did not differ in current users of DPP-4 inhibitors and non-diabetic controls, but the follow-up time was probably too short to detect an effect [47].

Finally, a cohort study from South Korea suggested a protective effect of DPP-4 inhibitors [48], whereas a post-hoc analysis of 20 randomized controlled trials found a slightly higher incidence of fractures with saxagliptin compared with control group [49]. In conclusion, clinical data are quite discordant and further studies are needed to define the real effect of DDP-4 inhibitors on human bone.

II. Effect of glucagon-like peptide 1 receptor agonists and dipeptidyl peptidase-4 inhibitors on bone turnover in postmenopausal women with type 2 diabetes

1. Aims

The primary objective of the study was the assessment of bone turnover in postmenopausal women affected with type 2 diabetes (T2DM) on treatment with dulaglutide, sitagliptin or other oral anti-diabetic drugs. The secondary objectives were the evaluation of the bone mineral density (BMD) and of morphometric vertebral fractures (VFx) in the same cohort of subjects.

2. Materials and methods

In this open, observational, prospective study all consecutive diabetic women referred to the outpatient clinic of the Endocrine-Metabolic Department of IRCCS Istituto Auxologico Italiano in Milan (Italy) have been evaluated for enrolment.

The exclusion criteria were: endocrine diseases (e.g. hyperthyroidism, primary hyperparathyroidism, hypercortisolism, idiopathic hypercalciuria, hyperandrogenism) or other diseases or conditions known to influence bone metabolism (e.g. rheumatoid arthritis, connective tissue diseases, malabsorption, intestinal bowel diseases, chronic liver disease, malignant neoplasia, alcoholism, depression, chronic obstructive pulmonary disease, multiple sclerosis or other severe motor impairment, a history of severe vitamin D deficiency), previous ketoacidosis, chronic diabetic complications, an ongoing treatment with drugs known to influence bone metabolism (e.g. glucocorticoids, antidepressants, anticonvulsants, thiazolidinediones, sodium–glucose cotransporter inhibitors, acarbose, insulin), a recent treatment with bone-active drugs (teriparatide or denosumab in the past 2 years and bisphosphonate or strontium ranelate in the past 5 years).

The inclusion criteria were: age ≥50 and <80 years; more than 5 years from menopause; T2DM diagnosis after 30 years of age; body mass index (BMI) ≥19 e <40 kg/m^2; stable weight (variation <5%) and stable diet in the previous 3 months.

According to these criteria, patients who started dulaglutide (arm 1), patients who started sitagliptin (arm 2) and patients who continued their own treatment or start metformin (arm 3), upon clinical judgement, were enrolled.

An informed consent, which was approved by the Local Ethical Committee and in accordance with the Declaration of Helsinki II, was obtained from all individual participants included in the study before entering the study.

From all the enrolled patients at the time of enrolment we collected information on familiar, physiological and medical history. Data regarding height, weight, waist and hip circumference were registered and, accordingly, BMI and waist-to-hip ratio were calculated. Validated questionnaires assessing falling risk [50], physical activity [51], sun exposure [52] and dietary calcium intake [53] were administered. In patients with a calcium intake <1000 mg/day an increase of dairy products consumption or an oral calcium carbonate supplementation (500 mg/day or 1000 mg/day in patients with an estimated calcium intake above or below 500 mg/day, respectively) was prescribed in order to reach recommended daily intake [54]. Moreover, at the time of enrolment, according to the current guidelines [55], in all patients we recommended a cholecalciferol supplementation (50000 UI at once in those not supplemented yet and then 25000 UI twice a month).

In those not supplemented yet the basal evaluation took place 15 days after the initial bolus of cholecalciferol. In all patients at the baseline and then after 6, 12 and 18 months the following parameters were measured: glycaemia, glycosylated haemoglobin (HbA1c), creatinine, alkaline phosphatase (ALP) and bone-specific ALP (bALP), calcium, phosphate, parathyroid hormone (PTH), 25-hydroxy vitamin D (25OHD), osteocalcin (OC), carboxy-terminal telopeptide of type I collagen (CTX), creatinine and calcium excretion in a 24-hour urine collection.

In all subjects the measurement of ALP, bALP, OC and CTX were carried out also 30 and 90 days after the baseline. Samples were collected in the morning after overnight fasting and stored at -20°C until assayed.

In all patients serum glycaemia, HbA1c, creatinine, ALP, calcium, phosphate, urinary calcium, phosphate and creatinine were measured by standard colorimetric techniques. Serum PTH and 25OHD

levels were measured by a chemiluminometric assay and bALP by a electrophoretic assay. Levels of OC and CTX were determined by ELISA immunoenzymatic assays.

In all patients at the baseline and after 18 months we evaluated BMD, body composition and the presence of morphometric VFx. Dual energy X-ray absorptiometry (DXA) scan will be carried out to measure BMD (Hologic Horizon A, Waltham, MA, USA) at lumbar spine (LS; in vivo precision 1.7%), total femur (FT; in vivo precision 1.7%), and femoral neck (FN; in vivo precision 1.8%). BMD data were expressed as Z-score and T-score (number of standard deviations above/below the mean for the patient's age, sex and ethnicity and above/below the mean for a healthy adult at the peak bone mass of the same sex and ethnicity as the patient, respectively). BMD changes higher than the least significant change (LSC) [19] were considered statistically significant.

Vertebral fracture assessment (VFA) by DXA [9] was used in all subjects in order to detect morphometric VFx using the semiquantitative assessment previously described by Genant [10]. Two trained physicians, blinded to BMD and biochemical data, independently reviewed the images, discussing questionable cases to agree on a diagnosis.

An additional conventional spinal radiograph in lateral and anteroposterior projection with standardized technique was obtained just in case of two or more unevaluable vertebrae, poor visualization due to moderate to severe scoliosis or a need to confirm possible mild vertebral fracture [56].

Body composition was assessed using Akern impedance analysers and the application BodygramPlus Enterprise 1.1.0.19 (Akern S.r.l., Italy) for data processing. Fat Mass (FM) and Fat Free Mass (FFM) were calculated.

Hypothesizing a mean variation of bone turnover markers of 15% in patients treated with dulaglutide (arm 1) or sitagliptin (arm 2) and no variation in the remaining patients (arm 3) and a standard deviation up to 20% about 90 experimental subjects would have been needed (30 for each arm) to ensure a 90% power and a 5% Type I Error.

Statistical analysis was performed by SPSS version 24.0 statistical package (SPSS Inc, Chicago, IL). The results were expressed as median values and ranges or absolute number and percentage.

3. Results

Unfortunately, the present study protocol has been seriously limited by COVID19 pandemic.

In 2020 the interruption of non-urgent outpatient clinical activity due to the sanitary emergency prevented the recruitment of new subjects in the study and the execution of the follow-up visits according to the timing provided by the protocol.

Moreover, even in the subsequent months when the restrictions were loosened, many subjects refused the inclusion in the study or put off the scheduled visits because of the fear of contagion.

Eventually, according to the exclusion and inclusion criteria, 8 T2DM postmenopausal women were recruited: 2 who started dulaglutide (arm 1), 2 who started sitagliptin (arm 2) and 4 who continued their own treatment (arm 3). Only 3 subjects (2 and 1 belonging to the arm 1 and 3, respectively) completed the 18-month follow-up, whereas the remaining 5 subjects put off some of the follow-up visits and did not completed the study protocol.

The clinical and biochemical characteristics of the recruited patients at baseline are reported in Table 1.

No patients were current smokers. Three patients were overweight and the remaining were obese (3 mild, 2 moderate). No patients had T2DM-related complications and presented a high risk of falling [50]. All patients were already on treatment with metformin before entering the study. Almost all of them (7/8, 87.5%) suffered from hypertension and the whole sample was dyslipidaemic, although only 5 out of 8 were on treatment with statins.

Only 2 patients (25%) were already on cholecalciferol supplementation before entering the study, whereas the others started taking vitamin D after the recruitment. After the cholecalciferol supplementation provided by the protocol, at baseline only 2 patients (25%) were able to reach normal vitamin D levels (≥30 μg/L). However, all patients achieved the threshold of 20 μg/L. At the baseline evaluation 2 patients (25%) had a condition of secondary hyperparathyroidism which normalized in the following follow-up visits.

Three patients (37.5%) were osteoporotic according to WHO criteria (T-score ≤-2.5), but this number rise to 4 (50%) if considered a T-score equal or lower than -2.0, the proposed BMD intervention threshold in T2DM patients [18, 57].

Two patients reported a previous clinical fragility fracture, in both cases at the humerus. At baseline these patients were not osteoporotic according to BMD values (one of the two showed a FN T-score lower than -2.0). No patients experienced a previous major clinical fragility fracture (hip or vertebral).

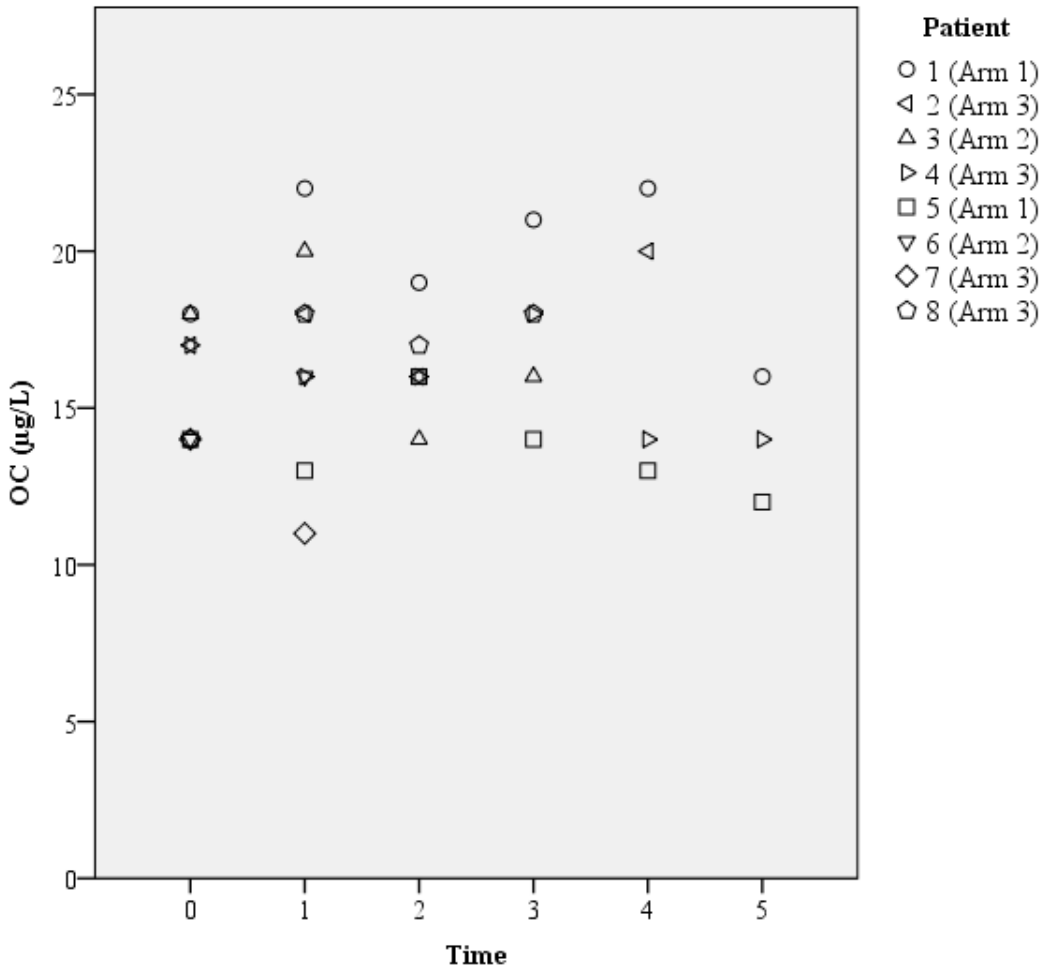
The trend of OC, CTX and bALP is depicted in Figures 3-4-5, respectively.

Table 1. Clinical and biochemical characteristics of the recruited T2DM patients at baseline

Parameters	**T2DM patients (n=8)**
Age (years)	67.0 (56−76)
Years from menopause	12.5 (4−23)
T2DM duration (years)	7.5 (4−17)
BMI (kg/m^2)	32.87 (25.6−38.9)
Waist-to-hip ratio	0.95 (0.9−1.0)
FM (%)	44.75 (24.9−52.4)
FFM (%)	55.25 (47.6-65.1)
Mean HbA1c (mmol/mol)	54 (48−61)
Mean daily calcium intake (mg/day)[a]	960.4 (612.0−1084.2)
Sun Exposure Score[b]	14.0 (6−28)
Level of physical activity (low/moderate/high)[c]	1/5/2 (12.5/62.5/25.0)
LS BMD (Z-score)	-0.50 (-0.90−3.70)
FN BMD (Z-score)	0.20 (-0.40−1.40)
FT BMD (Z-score)	0.70 (-0.40−2.50)
Prevalence of BMD T-score ≤-2.5/≤-2.0	3/4 (37.5/50.0)
Prevalence of clinical fragility fractures	2 (25.0)
Prevalence of morphometric vertebral fragility fractures	1 (12.5)
Prevalence of fragility fractures	2 (25.0)
Calcium (mg/dL)	9.80 (9.60−10.4)
Phosphorus (mg/dL)	3.60 (3.10−4.40)
PTH (ng/L)	38.65 (17.70−78.90)
25-hydroxy vitamin D (µg/L)	27.50 (25.0−43.10)
OC (µg/L)	15.50 (14.0−18.0)
CTX (ng/L)	313.50 (274−394)
ALP (U/L)	84.0 (58−103)
bALP (µg/L)	17.15 (12.1−21.6)
Creatinine (mg/dL)	0.77 (0.46−1.11)
Urinary calcium excretion (mg/kg/day)	1.07 (0.30−3.90)

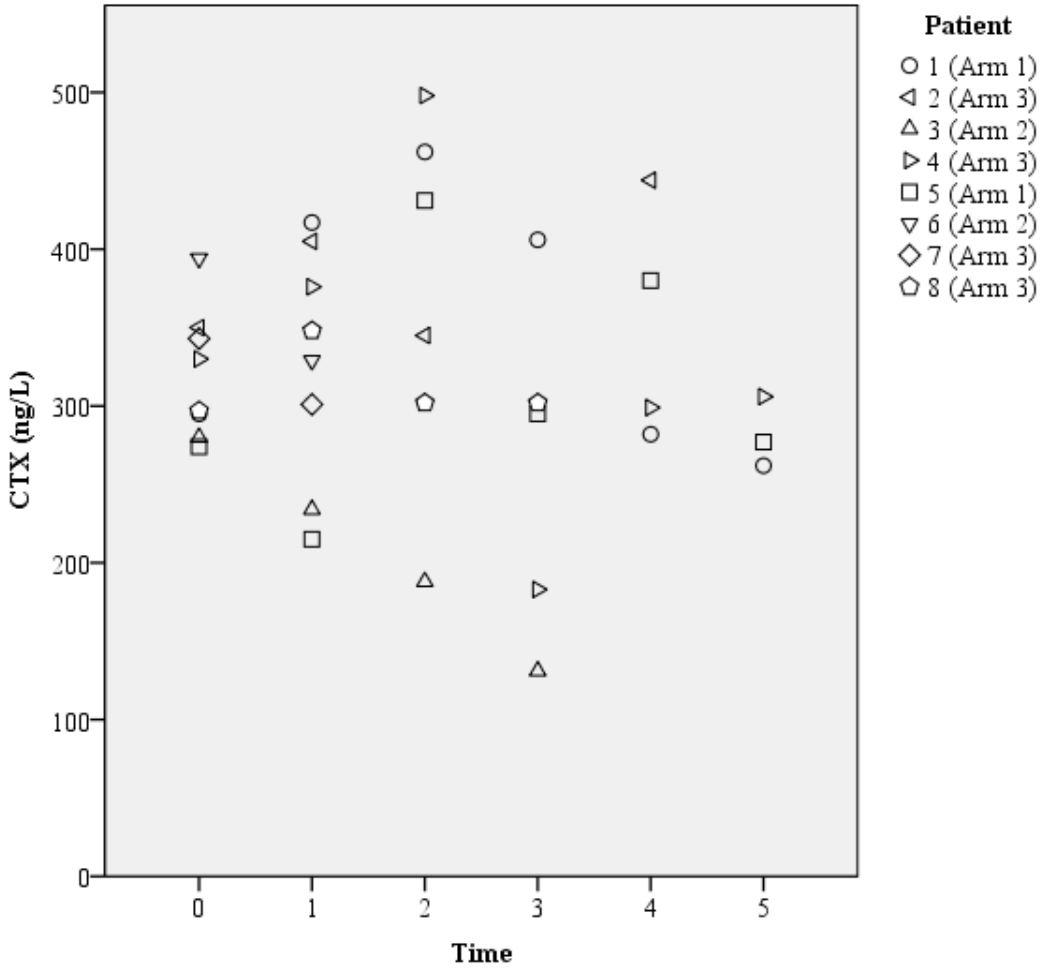
Data are expressed as median values with ranges in parentheses or absolute number with percentage in parentheses. T2DM: type 2 diabetes mellitus. BMI: Body Mass Index. FM: Fat Mass. FFM: Fat Free Mass. HbA1c: glycosylated haemoglobin. BMD: bone mineral density. LS: lumbar spine. FN: femoral neck. FT: femur total. PTH: parathyroid hormone (normal values n.v. 13.0-64.0 ng/L). OC: osteocalcin (n.v.: 5-59 µg/L). CTX: carboxy-terminal telopeptide of type I collagen (n.v. <1008 ng/L). ALP: alkaline phosphatase (n.v. 35-105 U/L). bALP: bone-specific ALP (n.v. 10.0-42.0 µg/L). [a, b, c]: assessed with validated questionnaires ([51–53], respectively).

Figure 3. Osteocalcin (OC) levels (normal values: 5-59 µg/L)



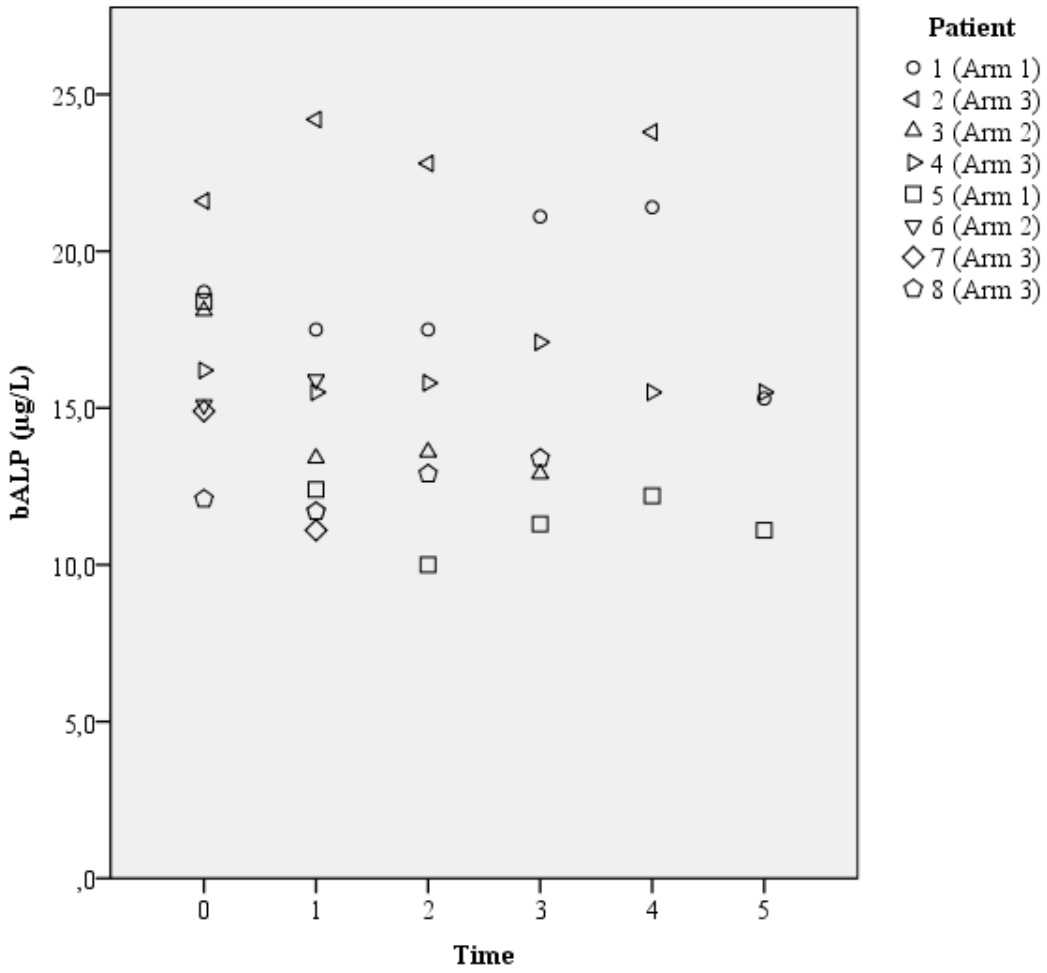
Arm 1: patients who started dulaglutide. Arm 2: patients who started sitagliptin. Arm 3: patients who continued their own treatment. Time 0: baseline. Time 1: 1-month follow-up. Time 2: 3-month follow-up. Time 3: 6-month follow-up. Time 4: 12-month follow-up. Time 5: 18-month follow-up.

Figure 4. Carboxy-terminal telopeptide of type I collagen (CTX) levels (normal values <1008 ng/L)



Arm 1: patients who started dulaglutide. Arm 2: patients who started sitagliptin. Arm 3: patients who continued their own treatment. Time 0: baseline. Time 1: 1-month follow-up. Time 2: 3-month follow-up. Time 3: 6-month follow-up. Time 4: 12-month follow-up. Time 5: 18-month follow-up.

Figure 5. Bone-specific alkaline phosphatase (bALP) levels (normal values 35-105 U/L)



Arm 1: patients who started dulaglutide. Arm 2: patients who started sitagliptin. Arm 3: patients who continued their own treatment. Time 0: baseline. Time 1: 1-month follow-up. Time 2: 3-month follow-up. Time 3: 6-month follow-up. Time 4: 12-month follow-up. Time 5: 18-month follow-up.

The 18-month follow-up data of the patients who completed the study protocol are summarized in Table 2. In all patients the OC, CTX and bALP levels at the end of follow-up showed variations lower than the least significant change (LSC) [58]. Even the BMD values after 18 months were stable as compared to baseline ones, except for LS BMD of patient 1 which exhibited a statistically significant increase. However, the same patient is the one who presented an incident morphometric VFx. No clinical fractures occurred during the protocol.

Table 2. Clinical and biochemical characteristics of the T2DM patients who completed the 18-month study protocol

Patient	ΔOC (%)	ΔCTX (%)	ΔbALP (%)	ΔLS BMD (%)	ΔFN BMD (%)	ΔFT BMD (%)	Incident Fx
1 (Arm 1)	-11.1	-11.2	-18.2	6.5*	0.8	-3.6	Yes Morphometric
4 (Arm 3)	-17.6	-9.4	-4.3	0.3	-3.9	-1.4	No
5 (Arm 1)	1.1	-14.3	-15.2	-4.5	1.7	-0.3	No

Arm 1: patients who started dulaglutide. Arm 3: patients who continued their own treatment. Δ: percentage change versus baseline. OC: osteocalcin. CTX: carboxy-terminal telopeptide of type I collagen. bALP: bone-specific alkaline phosphatase. LS: Lumbar Spine. FN: Femoral Neck. FT: Femur Total. Fx: fracture. *: statistically significant change.

4. Discussion

The main limitation of the present protocol is the low sample size which does not consent to perform statistical analysis on follow-up data and to draw conclusions about the effect of glucagon-like peptide 1 (GLP-1) receptor agonists and dipeptidyl peptidase-4 inhibitors (DPP-4) inhibitors on bone turnover, BMD and fracture risk in T2DM patients.

However, these preliminary data on a very limited sample of postmenopausal T2DM patients allow to make some observations.

The current sample confirmed the low bone turnover state of T2DM postmenopausal women [14, 15]. At baseline the bone markers of both formation (bALP and OC) and resorption (CTX) placed in the lower half of the reference range. No patients showed values that exceeded this threshold, despite the compresence in 2 patients of a condition of secondary hyperparathyroidism.

On the basis of the available data, the presence of a low bone turnover did not seem to change after treatment: all values of bone turnover markers stayed in the lower half of the reference range even in the follow-up visits, regardless of the arm of treatment. Three patients who completed the protocol (two belonging to arm 1 who were treated with dulaglutide and one belonging to arm 3 who continued metformin) showed OC, CTX and bALP values after 18-month follow-up which were comparable to the baseline levels since measured variations did not exceed the LSC.

The information derived from this study also supported the limited value of DXA in the prediction of fracture risk in T2DM patients [2, 4]. Both patients who reported a previous clinical fragility fracture had a BMD T-score not in the osteoporotic range (and only one of the two lower than the threshold of -2.0). Moreover, a morphometric vertebral fracture was identified in the course of follow-up in the one patient who showed a significant increase of the BMD at the spine, thus probably due to a worsening of osteoarthritis rather than to an improvement of bone status.

The very low number of recruited subjects does not consent to obtain conclusive information on the effect of GLP-1 receptor agonists on fracture risk Indeed, the occurrence of an incident VFx, although not clinical, in a patient belonging to arm 1 (on treatment with dulaglutide) assumes an anecdotic value.

Previous studies on this topic gave discordant results [35, 40–44] and then further studies are needed to clarify the effect of incretin-based therapies on bone turnover and fracture risk. The continuation of this study protocol with the sample expansion could help to clarify the relationship between GLP-1 receptor agonists and DDP-4 inhibitors and bone health in T2DM patients.

Since both osteoporosis and T2DM are important healthcare issues, the potential availability of anti-diabetic drugs able to reduce fracture risk could allow to lower costs linked to bone-active treatments and to fracture-related comorbidities in diabetic patients.

III. Validation of the clinical consensus recommendations on bone health assessment and management of fracture risk in postmenopausal women with type 2 diabetes

1. Aims

The aim of the present study was to validate the clinical consensus recommendations on the assessment and management of fracture (Fx) risk in patients with type 2 diabetes (T2DM) established by the Italian multidisciplinary expert panel [18] and in particular to assess, on the basis of such diagnostic approaches: i) how many T2DM patients with clinical and/or asymptomatic vertebral fracture (VFx) and how many T2DM patients without Fx would have been pharmacologically treated; ii) whether T2DM patients who would have been treated were really at high risk of Fx and, conversely, those who would have been conservatively followed up, were really at low risk for Fx.

2. Materials and methods

The present study is the continuation of a previous protocol aimed to evaluate bone involvement in a group of diabetic postmenopausal female subjects with T2DM [7]. They were selected on the basis of the following criteria: age 50-80 years, post-menopausal status, T2DM diagnosis after 30 years of age; BMI 19-40 kg/m^2, glycosylated haemoglobin (HbA1c) ≤64 mmol/mol. The exclusion criteria were the following: insulin therapy during the first 2 years of the disease, history of ketoacidosis or hypoglycaemia in the past 6 months before enrolment; an ongoing treatment with drugs known to influence bone metabolism (e.g. glucocorticoids, antidepressants, anticonvulsants, thiazolidinediones, sodium–glucose cotransporter inhibitors, acarbose), a recent treatment with bone-active drugs (teriparatide or denosumab in the past 2 years and bisphosphonate or strontium ranelate in the past 5 years); endocrine diseases (e.g. hyperthyroidism, primary hyperparathyroidism, hypercortisolism, idiopathic hypercalciuria, hyperandrogenism) or other diseases or conditions known to influence bone metabolism (e.g. rheumatoid arthritis, connective tissue diseases, malabsorption, intestinal bowel diseases, chronic liver disease, malignant neoplasia, alcoholism, depression, chronic obstructive pulmonary disease, multiple sclerosis or other severe motor impairment, a history of severe vitamin D deficiency); presence of proliferative or laser-treated retinopathy, overt diabetic nephropathy (macroalbuminuria >300 mg/24-hour), severe macroangiopathy (history of myocardial infarction,

coronary artery bypass graft surgery, percutaneous transluminal coronary, carotid, femoral or femoral-popliteus angioplasty).

Eventually, at baseline our sample was formed by 107 Caucasian female subjects with T2DM (99 patients enrolled in the original protocol [7] and 8 patients described before). At the basal evaluation the presence of previous fragility Fx and of the major T2DM-specific Fx risk factors according to the guidelines (a disease duration above 10 years, the presence of one or more chronic T2DM complications, the use of insulin or thiazolidinediones and persistent poor glycaemic control i.e. HbA1c levels above 64 mmol/mol for at least 1 year) [18] was ascertained by the clinical history and by the review of the medical reports.

In keeping with the expert panel [18], if requested by the flow-chart, the WHO Fracture Risk Assessment algorithm (FRAX) was calculated without bone mineral density (BMD) and with rheumatoid arthritis as surrogate risk factor of diabetes [59]. A T2DM patient was considered FRAX+ if fulfilled the National Osteoporosis Foundation criteria for treatment: 20% ten years risk of major fragility Fx and 3% ten years risk of hip Fx [60].

Enrolled patients were conservatively followed-up. At the baseline and then after a minimum follow-up of 18 months we evaluated BMD and the presence of morphometric VFx. Dual energy X-ray absorptiometry (DXA) scan will be carried out to measure BMD (Hologic Discovery or Horizon A, Waltham, MA, USA) at lumbar spine (LS; in vivo precision 1.7%), total femur (FT; in vivo precision 1.7%), and femoral neck (FN; in vivo precision 1.8%), whereas vertebral fracture assessment (VFA) by DXA [9] or a conventional spinal radiograph in lateral and anteroposterior projection with standardized technique were used to detect morphometric vertebral fractures using the semiquantitative assessment previously described by Genant [10]. Two trained physicians, blinded to BMD and biochemical data, independently reviewed the images, discussing questionable cases to agree on a diagnosis. According to the perspective by Chiodini and coauthors [18] only moderate and severe VFx were included in the analysis.

Those patients who developed during the follow-up one of the exclusion criteria were excluded from the follow-up analysis.

The BMD at the follow-up was considered increased or decreased if the BMD variations were respectively higher or lower than the least significant change (LSC) in at least one skeletal site.

Finally, the incidence of clinical fragility Fx during the follow-up was recorded and confirmed by the review of the medical reports.

The study was designed to answer the following questions: if the protocol would have been applied to our sample of patients:

i. How many T2DM patients with clinical Fx would have been pharmacologically treated?
ii. How many T2DM patients without clinical Fx would have been pharmacologically treated?
iii. How many T2DM patients with asymptomatic VFx would not have been pharmacologically treated and how many patients without both clinical and morphometric VFx would have been pharmacologically treated?
iv. How many T2DM patients with at least 1 major T2DM-related risk factor for Fx, but without both clinical Fx and morphometric VFx would have been pharmacologically or conservatively treated on the basis of the FRAX score and/or BMD levels?
v. How many T2DM patients experienced a fragility Fx during the follow-up and who of them would have been pharmacologically treated?

Statistical analysis was performed by SPSS version 24.0 statistical package (SPSS Inc, Chicago, IL). The results were expressed as mean ± standard deviation (SD) with range in parentheses or absolute number with percentage in parentheses. The normality of distribution was tested by Kolmogorov–Smirnov test. The comparison of continuous variables between groups were performed using Student's t-test or Mann–Whitney U test as appropriate. Categorical variables were compared by $\chi 2$ test or Fisher Exact test, as appropriate.

A pooled odds ratio (OR) and the corresponding 95% confidence interval (CI) was obtained according to the random effect method proposed by Der Simonian and Laird [61].

P-values of less than 0.05 were considered significant.

3. Results

General overview

Following the flow-charts depicted in Figure 2, in our sample of 107 T2DM patients, 34 (31.8%) and 73 (68.2%) would have been pharmacologically and conservatively treated, respectively. The comparison between the characteristics of T2DM patients who would have been pharmacologically treated and those of patients who would have been conservatively followed-up is reported in Table 3. As compared with patients who would not have been pharmacologically treated, T2DM subjects who would have been treated were older, had a lower BMD at the femur and had a higher prevalence of asymptomatic VFx, low BMD (T-score ≤ -2.0 in at least one skeletal site), insulin treatment, T2DM duration above 10 years, neuropathy, at least one T2DM-related chronic complication and at least one major T2DM-related risk factor for Fx. In patients who would have been pharmacologically treated the BMD at the spine tended to be lower than in patients who would not have been pharmacologically treated, despite not reaching the statistical significance. The two groups were comparable as far as BMI, HbA1c levels, prevalence of retinopathy and nephropathy, even if these T2DM chronic complications were 3-4 fold more frequent in patients who would have been pharmacologically treated.

How many T2DM patients with clinical fractures would have been pharmacologically treated?

Following the flow-chart depicted in Figure 2a, among clinically fractured patients (19 out of 107, 17.8%), we would have pharmacologically treated one patient with hip Fx and, among the 18 patients with a clinical non-hip (i.e. vertebral, humerus or wrist) Fx, we would have treated 7 patients with at least one moderate or severe VFx, 3 patients without VFx but with BMD T-score ≤ -2.0, and 3 patients without VFx and without BMD T-score ≤ -2.0 but with FRAX+. Overall 14 out of 19 patients (73.7%) with clinical Fx would have been pharmacologically treated.

On the other hand, 5 patients (26.3%) with a clinical fragility Fx would not have been treated since they did not show asymptomatic VFx, had a BMD T-score above -2.0 and were at low risk by FRAX score. Among these patients, in keeping with a low-risk Fx profile in spite of the presence of a

fragility Fx, no one had T2DM complications, no one was on insulin treatment and only one showed a T2DM duration above 10 years.

The comparisons between the clinical characteristics of T2DM patients with clinical Fx and those without clinical Fx and between T2DM patients with clinical and/or asymptomatic vertebral Fx and those without Fx are reported in Table 4.

As compared to patients without clinical Fx, T2DM patients with clinical Fx showed a lower BMD at the FN and an increased prevalence of asymptomatic moderate or severe VFx, retinopathy, nephropathy and neuropathy. Moreover, as expected, the prevalence of patients with at least one T2DM-related chronic complication was increased in clinically fractured patients than in patients without clinical Fx. Age, BMI, HbA1c levels, spine BMD and the prevalence of T2DM duration above 10 years, insulin treated subjects and patients with at least one major T2DM-related risk factor for Fx were comparable between the two groups.

As compared to patients without Fx, patients with clinical and/or morphometric moderate or severe VFx showed an increased prevalence of nephropathy and neuropathy. Moreover, they also showed a higher prevalence of subjects with insulin treatment, with a T2DM duration longer than 10 years, with at least one major T2DM-related risk factor for Fx and with at least one T2DM chronic complication. Age, BMI, BMD and HbA1c levels were comparable between the two groups.

How many T2DM patients without clinical fractures would have been pharmacologically treated?

Following the flow-chart depicted in Figure 2b, among the 88 patients without clinical Fx, we would have pharmacologically treated 20 (22.7%) patients: 9 patients with at least one morphometric moderate or severe VFx, 8 patients without morphometric VFx but with a BMD T-score $\leq$ -2.0, 3 patients without a morphometric VFx but with a FRAX score suggesting a high Fx risk regardless of BMD. On the other hand, 68 patients (77.3%) would have been conservatively followed up: 49 patients without a major T2DM-related risk factor for Fx, 10 patients with at least 1 major T2DM-related risk factor for Fx but without VFx and with a negative FRAX score regardless of BMD and 9 patients with BMD T-score above -2.0 and with a FRAX score suggesting low Fx risk.

How many T2DM patients with asymptomatic VFx would not have been pharmacologically treated and how many patients without both clinical and morphometric VFx would have been pharmacologically treated?

Among the overall group of 73 patients without treatment indication, only 1 moderate morphometric VFx was present at the spinal evaluation. In other words, in our sample, among patients with asymptomatic VFx (17 out of 107, 15.9%), following the flow-charts depicted in Figure 2, 16 would have been pharmacologically treated (94.1%) and only one would have not been pharmacologically treated because of the absence of previous clinical Fx and of major T2DM-related risk factors and, thus, no indication to vertebral Fx assessment according to the diagnostic approach proposed by the expert panel [18].

Conversely, among the overall group of 34 patients would have been pharmacologically treated, 11 patients (32.4%) had neither clinical nor morphometric VFx and received the treatment indication according to the presence of at least one major T2DM-related risk factors and a T-score ≤-2.0 (n=7) or a FRAX+ (n=4).

How many T2DM patients with at least 1 major T2DM-related risk factor for fracture, but without both clinical Fx and morphometricVFx would have been pharmacologically or conservatively treated on the basis of the FRAX score and/or BMD levels?

Among the 30 patients without both clinical Fx and morphometric VFx but with at least 1 major T2DM-related risk factor for Fx, 13 had not the ISCD (International Society of Clinical Densitometry) indication for performing a DXA exam [19]. Among these latter, 11 patients were at low Fx risk based on the FRAX score and, thus, would be conservatively treated, while 2 patients, who were found to be at high risk for Fx on the basis of the FRAX score, would have been pharmacologically treated.

On the other hand, among the same 30 patients, 17 had the ISCD indication for performing a DXA exam [19]. Among these latter, 7 patients showing a BMD T-score equal or below -2.0 and 2 patients showing a BMD T-score above -2.0 but a high risk of Fx on the basis of the FRAX score, would be pharmacologically treated. Eight patients, showing both a BMD above -2.0 and being at low Fx risk by FRAX score, would have been conservatively treated.

The comparisons between the clinical characteristics of T2DM patients without both clinical Fx and major T2DM-related risk factors and T2DM patients without clinical Fx but with at least one major T2DM-related risk factor for Fx is reported in Table 5.

As compared with patients without both clinical Fx and major T2DM-related risk factors, patients without clinical Fx but with ≥1 major T2DM-related risk factor for Fx showed an increased prevalence of asymptomatic VFx and, as expected, an increased prevalence of T2DM duration above 10 years and insulin treatment. No statistically significant differences were found as far as age, BMI, HbA1c levels, BMD at both spine and femur and prevalence of T2DM-related chronic complications and T-score ≤-2.0. Importantly, among the 49 patients without both clinical Fx and major T2DM-related risk factors, who would have been, therefore, conservatively followed up, only one subject (2.0%, sensitivity 90%, negative predictive value NPV 98%), in fact, showed a prevalent VFx, while among the 39 patients without clinical Fx but with at least 1 major T2DM-related risk factors 9 subjects (23.1%, specificity 61.5%) showed a VFx and then they would have been pharmacologically treated, but 11 out of the remaining 30 would have received the treatment indication, anyway, according to the BMD T-score or to the FRAX score.

How many T2DM patients experienced a fragility fracture during the follow-up and who of them would have been pharmacologically treated?

Among the 107 patients evaluated at baseline, 11 patients were excluded from the longitudinal arm of the study because of the development in the course of follow-up of one of exclusion criteria provided by the protocol. Among the remaining, a follow-up was available for 31 patients. The median follow-up was of 55.5 months (range 18-100).

Following the flow-charts depicted in Figure 2, 13 out of these 31 T2DM patients would have been pharmacologically treated: 3 patients reported a previous clinical non-vertebral and non-hip fragility Fx associated with another factor which justified the treatment indication (one had a moderate morphometric VFx, one a BMD T-score ≤-2.0 and one a FRAX score suggesting a high Fx risk), 7 patients had at least one major T2DM-related risk factor and at least one morphometric VFx and finally 3 had at least one major T2DM-related risk factor and a BMD T-score ≤ -2.0.

At the follow-up evaluation 21 patients (67.7%) showed no significant BMD variations. In 4 patients (12.9%) BMD was significantly increased, whereas in 6 patients (19.4%) was significantly decreased. The BMD variations according to the baseline treatment indication are summarized in Table 6. It is noteworthy that in all 4 cases of BMD gain the improvement interested the lumbar spine and not the femoral site.

During the follow-up period two patients out of 31 experienced an incident fragility Fx (6.5%): one patient had a clinical vertebral Fx and one a morphometric moderate VFx. In both cases vertebral fractures involved the dorsal tract. The incidence rate was 15 Fx per 1000 patient-years.

Both patients with incident Fx would have been pharmacologically treated at baseline (Table 6) on the basis of the compresence of at least one major T2DM-related factor risk and a BMD T-score ≤-2.0. The clinical characteristics of these 2 patients are summarized in Table 7. At the follow-up evaluation one patient showed no significant BMD variations, whereas paradoxically the other experienced a significant BMD gain at the vertebral site.

Based on these data, among our T2DM patients with treatment indication according to the clinical recommendations established by the Italian multidisciplinary expert panel [18] the incident rate was 30 Fx per 1000 patient-years. A T2DM patient with bone-active treatment indication tended to have an 8-fold increased risk of experiencing an incident Fx in the subsequent years (OR 8.33, 95%I.C: 0.36-190.9, p=0.095) compared to patients without this indication, although not reaching the statistical significance probably because of the low number of incident Fx.

Table 3. Characteristics of T2DM patients who would have been pharmacologically treated and of patients who would have been conservatively followed-up

Parameters	**T2DM patients who would have been pharmacologically treated (n=34)**	**T2DM patients who would not have been pharmacologically treated (n=73)**	***p***
Age (years)	68.5±7.1 (53–80)	64.3±7.0 (52–80)	**0.005**
BMI (kg/m^2)	30.1±4.3 (21.0–40.0)	29.4±5.1 (21.3–40.0)	0.485
HbA1c (mmol/mol)	53±8 (40–64)	50±8 (31–64)	0.113
Patients with asymptomatic VFx (moderate or severe)	16 (47.1)	1 (1.4)	**<0.0001**
Patients with T2DM duration ≥10 years	26 (76.5)	19 (26.0)	**<0.0001**
Patients on insulin treatment	10 (29.4)	10 (13.7)	**0.05**
Patients with retinopathy	3 (8.8)	2 (2.7)	0.182
Patients with nephropathy	4 (11.8)	2 (2.7)	0.079
Patients with neuropathy	4 (11.8)	0 (0.0)	**0.009**
Patients with at least one T2DM chronic complication[a]	6 (17.6)	3 (4.1)	**0.028**
Patients with at least 1 major T2DM-related risk factor for Fx[b]	31 (91.2)	20 (27.4)	**<0.0001**
Patients with BMD T-score ≤-2.0	20 (58.8)	14 (19.2)	**<0.0001**
LS BMD (T-score)	-1.21±1.45 (-3.60–1.50)	-0.67±1.41 (-5.30–2.90)	0.068
FN BMD (T-score)	-1.65±0.92 (-3.20–0.70)	-0.85±1.03 (-3.40–1.40)	**<0.0001**

Data are mean±SD with range in parentheses or absolute number with percentage in parentheses. T2DM: type 2 diabetes. BMI: body mass index. HbA1c: glycosylated haemoglobin. BMD: bone mineral density; LS: lumbar spine. FN: femoral neck. VFx: vertebral fracture (moderate or severe). Fx: fracture. [a]: patients with at least one out of nephropathy, retinopathy and neuropathy. [b]: patients with at least one out of a disease duration above 10 years, the presence of one or more chronic T2DM complications, the use of insulin or thiazolidinediones and persistent poor glycaemic control (i.e. HbA1c levels above 64 mmol/mol for at least 1 year).

Table 4. Comparisons between the clinical characteristics of T2DM patients with clinical fractures and those without clinical fractures and between T2DM patients with clinical and/or asymptomatic vertebral fracture and those of T2DM patients without fractures

Parameters	**All T2DM patients (n=107)**	**T2DM patients with clinical Fx (n=19)**	**T2DM subjects without clinical Fx (n=88)**	***p*[1]**	**T2DM patients with clinical Fx and/or asymptomatic VFx (n=29)**	**T2DM patients without clinical Fx and asymptomatic VFx (n=78)**	***p*[2]**
Age (years)	65.6±7.3 (52–80)	66.5± 7.9 (54–77)	65.5±7.2 (52–80)	0.587	65.8±8.0 (52–77)	65.6±7.1 (52–80)	0.899
BMI (kg/m^2)	29.6±4.8 (21.0–40.0)	29.8±3.9 (24.2–40.0)	29.6±5.0 (21.0–40.0)	0.877	30.1±3.8 (23.3–40.0)	29.5±5.2 (21.0–40.0)	0.535
HbA1c (mmol/mol)	51±8 (31–64)	52±8 (40–64)	51±8 (31–64)	0.625	52±7 (40–64)	50±9 (31–64)	0.257
Patients with asymptomatic VFx (moderate or severe)	17 (15.9)	7 (36.8)	10 (11.4)	**0.006**	17 (58.6)	0 (0.0)	**<0.0001**
Patients with T2DM duration ≥10 years	45 (42.1)	9 (47.4)	36 (40.9)	0.605	17 (58.6)	28 (35.9)	**0.034**
Patients on insulin treatment	20 (18.7)	4 (21.1)	16 (18.2)	0.752	9 (31.0)	11 (14.1)	**0.046**
Patients with retinopathy	5 (4.7)	3 (15.8)	2 (2.3)	**0.038**	3 (10.3)	2 (2.6)	0.122
Patients with nephropathy	6 (5.6)	4 (21.1)	2 (2.3)	**0.009**	4 (13.8)	2 (2.6)	**0.045**
Patients with neuropathy	4 (3.7)	4 (21.1)	0 (0.0)	**0.001**	4 (13.8)	0 (0.0)	**0.005**
Patients with at least one T2DM chronic complication[a]	9 (8.4)	6 (31.6)	3 (3.4)	**0.001**	6 (20.7)	3 (3.8)	**0.011**
Patients with at least 1 major T2DM-related risk factor for Fx[b]	51 (47.7)	12 (63.2)	39 (44.3)	0.136	21 (72.4)	30 (38.5)	**0.002**
Patients with BMD T-score ≤-2.0	34 (31.8)	9 (47.4)	25 (28.4)	0.107	11 (37.9)	23 (29.5)	0.404
LS BMD (T-score)	-0.84±1.44 (-5.30–2.90)	-1.18±1.47 (-3.20–1-50)	-0.76±1.43 (-5.30–2.90)	0.255	-0.89±1.36 (-3.20–1.50)	-0.82±1.48 (-5.30–2.90)	0.140
FN BMD (T-score)	-1.11±1.06 (-3.40–1.40)	-1.56±0.89 (-3.20–0.30)	-1.01±1.07 (-3.40–1.40)	**0.038**	-1.36±0.89 (-3.20–0.70)	-1.01±1.11 (-3.40–1.40)	0.169

Data are mean±SD with range in parentheses or absolute number with percentage in parentheses.
[1]p=levels of statistical significance betweenT2DM patients with clinical Fx (n=19) and T2DM subjects without clinical Fx (n=88). [2]p=levels of statistical significance between T2DM patients with clinical Fx and/or asymptomatic VFx (n=29) and T2DM patients without clinical Fx and asymptomatic VFx (n=78).
T2DM: type 2 diabetes. BMI: body mass index. HbA1c: glycosylated haemoglobin. VFx: vertebral fracture. Fx: fracture. BMD: bone mineral density. LS: lumbar spine. FN: femoral neck. [a]: patients with at least one out of nephropathy, retinopathy and neuropathy. [b]: patients with at least one out of a disease duration above 10 years, the presence of one or more chronic T2DM complications, the use of insulin or thiazolidinediones and persistent poor glycaemic control (i.e. HbA1c levels above 64 mmol/mol for at least 1 year).

Table 5. Comparisons between the clinical characteristics of T2DM patients without both clinical fractures and major T2DM-related risk factors and those of T2DM patients without clinical fractures but with at least one major T2D-related risk factor for fracture

Parameters	**All T2DM patients without clinical Fx (n=88)**	**T2DM patients without clinical Fx but with ≥1 major T2DM-related risk factor for Fx (n=39)**	**T2DM patients without both clinical Fx and major T2DM-related risk factor for Fx (n=49)**	***P*[1]**
Age (years)	65.6±7.2 (52–80)	65.8±7.6 (52–80)	65.2±6.9 (52–80)	0.726
BMI (kg/m^2)	29.6±5.0 (21.0–40.0)	29.8±4.3 (21.0–39.5)	29.4±5.5 (21.3–40.0)	0.737
HbA1c (mmol/mol)	51±8 (31–64)	52±8 (31–63)	50±8 (31–64)	0.390
Patients with asymptomatic VFx (moderate or severe)	10 (11.4)	9 (23.1)	1 (2.0)	**0.004**
Patients with T2DM duration ≥10 years	36 (40.9)	35 (89.7)	1 (2.0)	**<0.0001**
Patients on insulin treatment	16 (18.2)	14 (35.9)	2 (4.1)	**<0.0001**
Patients with retinopathy	2 (2.3)	2 (5.1)	0 (0.0)	0.194
Patients with nephropathy	2 (2.3)	1 (2.6)	1 (2.0)	0.693
Patients with neuropathy	0 (0.0)	0 (0.0)	0 (0.0)	-
Patients with at least one T2DM chronic complication[a]	3 (3.4)	2 (5.1)	1 (2.0)	0.582
Patients with BMD T-score ≤-2.0	25 (28.4)	11 (28.2)	14 (28.6)	0.970
LS BMD (T-score)	-0.76±1.43 (-5.30–2.90)	-0.86±1.30 (-3.6–2.5)	-0.69±1.54 (-5.30–2.90)	0.595
FN BMD (T-score)	-1.01±1.07 (-3.40–1.40)	-1.08±1.12 (-2.9–1.00)	-0.95±1.04 (-3.40–1.40)	0.567

Data are mean±SD with range in parentheses or absolute number with percentage in parentheses. [1]p=level of statistical significance between T2DM patients without clinical Fx but with ≥1 major T2DM-related risk factor for Fx (n=39) and T2DM patients without both without clinical Fx and major T2DM-related risk factor for Fx (n=49).
T2DM: type 2 diabetes. BMI: body mass index. HbA1c: glycosylated haemoglobin. BMD: bone mineral density. LS: lumbar spine. FN: femoral neck. Fx: fracture. VFx: vertebral fracture. [a]: patients with at least one out of nephropathy, retinopathy and neuropathy.

Table 6. BMD variations and incident fractures at follow-up in T2DM patients who would have been pharmacologically treated and who would have been conservatively followed-up

Follow-up parameters	All T2DM patients evaluated at follow-up (n=31)	T2DM patients who would have been pharmacologically treated (n=13)	T2DM patients who would not have been pharmacologically treated (n=18)	*p*
Patients with stable BMD	21 (67.4)	9 (69.2)	12 (66.7)	0.862
Patients with increased BMD	4 (12.9)	2 (15.4)	2 (11.1)	
Patients with decreased BMD	6 (19.4)	2 (15.4)	4 (22.2)	
Patients with incident fractures	2 (6.5)	2 (15.4)	0 (0.0)	0.168

Data are absolute number with percentage in parentheses. BMD: bone mineral density. T2DM: type 2 diabetes.

Table 7. Baseline characteristics of the T2DM patients with incident fractures at the follow-up evaluation

Baseline characteristics	Patient 1	Patient 2
Age (years)	65	67
BMI (kg/m^2)	28.7	32.9
Prevalent clinical fracture	No	No
Prevalent morphometric fracture	No	No
T2DM duration (years)	16	10
Insulin treatment	No	No
Retinopathy	No	No
Nephropathy	No	No
Neuropathy	No	No
Mean HbA1c (mmol/mol)	45	61
BMD LS (T-score)	-1.70	-2.50
BMD FN (T-score)	-2.90	-2.50
BMD FT (T-score)	-2.50	-1.90

T2DM: type 2 diabetes. BMI: body mass index. HbA1c: glycosylated haemoglobin. BMD: bone mineral density. LS: lumbar spine. FN: femoral neck. FT: femur total.

4. Discussion

Individuals with T2DM have an increased risk of bone fragility compared to the general population and diabetic patients with fragility Fx have higher mortality rates than individuals (diabetic or not) without Fx [62–64]. Bone fragility is then increasingly recognized as a complication of T2DM [1]. However, the underestimation of Fx risk in T2DM using the classical assessment tools [2, 4, 16, 17] pointed out the urgent need of clinical recommendations for the routine assessment of bone health in T2DM subjects. The clinical consensus recommendations formulated by the multidisciplinary expert panel chaired by Chiodini suggested two independent diagnostic approaches for the detection of T2DM patients worthy of bone-active treatment for the Fx risk reduction [18].

This study was aimed at applying these algorithms to our well characterized cohort of postmenopausal women with T2DM in order to validate them in clinical practice.

Following the flow-charts depicted in Figure 2, about one third of patients would have been pharmacologically treated (Table 3). As expected, the group of subjects with treatment indication showed a higher prevalence of risk factors of Fx, both those common to the general population (advanced age, prevalent asymptomatic VFx, low femoral BMD) and those disease-specific (insulin treatment, disease duration above 10 years, T2DM chronic complications considered as a whole). The lack of statistical difference in the glycaemic control between the two groups was due to the fact that, according to the inclusion criteria of our protocol, no recruited patients showed HbA1c levels higher than 64 mmol/mol and then a poor glycaemic control.

According to the consensus by Chiodini and collaborators, a previous non-vertebral, non-hip clinical Fx does not represent *per se* a criterion for treatment indication [18]. Among our T2DM patients with prevalent clinical fractures (who were only 19 out of 107), about three-quarters of them would have been pharmacologically treated, whereas the remaining quarter made up of 5 subjects would have been conservatively followed-up because of the absence of other criteria for active treatment (asymptomatic VFx, BMD T-score lower than -2.0 or FRAX+). Among these 5 patients only one had a major T2DM-related risk factors (she had been diabetic for more than 10 years,

whereas no one had T2DM chronic complications or was on insulin treatment), in keeping with a low risk of Fx profile despite the previous non-hip and non-vertebral clinical Fx.

According to the flow-chart illustrated in Figure 2b, among our T2DM patients without clinical Fx, nearly a quarter of them (20 out of 88) would have pharmacologically treated: in 9 patients the treatment indication came from the radiologic identification of morphometric moderate or severe VFx, which, despite being asymptomatic, are a known risk factor of subsequent Fx [8].

Considering all fractured patients together (both those with clinical Fx and those with morphometric VFx, Table 4), as expected fractured patients had a more advanced disease with a higher prevalence of each major T2DM-related factor risk than in patients without Fx, except for the poor glycaemic control due to the same reason mentioned above. Conversely, about a third of patients without any fractures received the treatment indication anyway, thus suggesting, despite the limited value of DXA scan and FRAX calculation in T2DM [2, 4, 17], the usefulness of these tools in selected cases.

It is interesting to observe that, through the application of the algorithms proposed by the multidisciplinary expert panel to our well-studied sample of postmenopausal women with T2DM [18], only one out of 17 patients with moderate or severe morphometric VFx, and then at established high risk of Fx, would not have been treated due to the lack of indication to vertebral fracture assessment and then the non-recognition of the prevalent morphometric VFx.

About this, from another point of view, among T2DM patients without clinical fractures the presence of at least one of the major T2DM-related risk factors for Fx showed a very good sensitivity in identifying who experienced a previous asymptomatic moderate or severe VFx and then was at increased risk of subsequent Fx [8]. In other words, the application of the flow-chart depicted in Figure 2 to our sample confirmed that the absence of both prevalent clinical fractures and major T2DM-related risk factor for Fx justifies the choice of a conservative follow-up without the radiological VFx assessment (NPV 98%).

Although on a limited fraction of patients, the follow-up data gave us the opportunity to validate the clinical consensus recommendations also in a prospective way.

Two incident Fx, both vertebral, occurred in our sample of 31 T2DM patients evaluated at follow-up. Notwithstanding the low number of followed-up subjects and incident Fx, it must be underlined that based on baseline data both fractured patients would have been pharmacologically treated if the diagnostic approach suggested by the expert panel [18] had been applied.

Conversely, no incident Fx were observed in the group of patients without any criteria for bone-active treatment indication, thus suggesting the ability of the algorithms by Chiodini and colleagues within the diabetic population to correctly detect subjects at relatively low risk of Fx despite the underlying disease. It is possible to postulate that the increased risk of incident Fx occurrence in patients with one of the criteria for active treatment indication compared to those without this indication did not probably reach the statistical significance because of the low number of analysed subjects and fracture events and an increase of sample size could overcome this limitation.

The application of the clinical consensus recommendations proposed by the expert panel to our sample confirmed the limited role of BMD in the monitoring of Fx risk in T2DM [2, 4]. The BMD variation at the follow-up examination in T2DM patients who would have been pharmacologically treated did not statistically differ from those observed in T2DM patients who would have been conservatively followed-up. Indeed, in about two thirds of patients bone mass showed no significant variations, independent of the basal Fx risk. Moreover, paradoxically an incident fragility morphometric VFx occurred exactly in a T2DM patient who experienced a BMD gain at the lumbar site. Precisely in this regard, it is noteworthy to underline that the BMD improvement at the follow-up DXA scan interested the lumbar site in 4 out of 4 cases. Then it is possible to hypothesize that in T2DM subjects the assessment and monitoring of LS BMD could not be reliable because of the common association of T2DM and osteoarthritis [65].

This study has some limitations: first of all, as already stated before, the small sample size of T2DM patients, especially those evaluated in the prospective arm, who were only a limited fraction of those included in the baseline assessment. An unwanted selection bias cannot be excluded for sure. Moreover, the variable length of follow-up can be also considered a limitation of this study because the shortest follow-up can have unavoidably underestimated the incidence of fragility Fx.

Furthermore, according to the exclusion and inclusion criteria provided by the protocol, our sample included neither some categories of T2DM patients at very high risk of Fx (e.g. patients on treatment with thiazolidinediones, with advanced T2DM chronic complications and with persistent poor glycaemic control i.e. HbA1c levels above 64 mmol/mol) nor male T2DM subjects.

Finally, mild morphometric VFx were not considered in the decision-making for treatment, but this is in line with the clinical consensus recommendations by Chiodini and colleagues [18] who excluded this kind of Fx from the assessment of Fx risk in order to reduce the possibility of incorrect classification of various vertebral deformities as VFx which could lead to unnecessary treatment prescription [66].

Nevertheless, despite these limitations, the clinical consensus recommendations established by the Italian multidisciplinary expert panel [18] performed well in our sample of postmenopausal women with T2DM. Indeed, among those subjects with bone-active treatment indication as many as 15.3% of patients experienced an incident Fx fracture during the follow-up, thus confirming the presence at baseline of a high Fx risk worthy of specific treatment and conversely, among those subjects without bone-active treatment indication no incident Fx were observed.

In the future, the application of the diagnostic approaches formulated by the multidisciplinary expert panel chaired by Chiodini [18] in a larger sample of T2DM patients with the inclusion also of subjects of both sexes and with a more severe disease (i.e. patients with proliferative or laser-treated retinopathy, overt diabetic nephropathy or severe macroangiopathy and patients with persistent HbA1c levels above 64 mmol/mol) could confirm the feasibility of the clinical consensus recommendations in the entire T2DM population. Clinical trials in those T2DM patients at risk for fragility Fx and deserving of bone-active treatment will then be needed to determine the actual efficacy and safety of available antiresorptive and anabolic agents in this specific setting.

IV. References

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