Title: Endogenous subclinical hypercortisolism and bone: a clinical review.

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Abstract

In the recent years the condition of subclinical hypercortisolism (SH) has become a topic of growing interest. This is due to the fact that the SH prevalence is not negligible (0.8%-2% in the general population) and that, although asymptomatic, this subtle cortisol excess is not harmless, being associated with an increased risk of complications, in particular of osteoporosis and fragility fractures. Since in SH the specific symptoms of hypercortisolism are absent, the SH diagnosis relies only on biochemical tests and it is a challenge for physicians. As a consequence, even the indications for the evaluation of bone involvement in SH patients are debated and guidelines are not available. Finally, the relative importance of bone density, bone quality and glucocorticoid sensitivity in SH is a recent field of research. On the other hand, the SH prevalence seems to be increased in osteoporotic patients, in whom a vertebral fracture may be the presenting symptom of an otherwise asymptomatic cortisol excess. Therefore, the issue of who and how to screen for SH among the osteoporotic patients is widely debated.

The present review will summarize the available data regarding the bone turnover, bone mineral density, bone quality and risk of fracture in patients with endogenous SH. In addition, the role of the individual glucocorticoid sensitivity in SH related bone damage and the problem of diagnosing and managing the bone consequences of SH will be reviewed. Finally, the issue of suspecting and screening for SH patients with apparent primary osteoporosis will be addressed.
Introduction

Subclinical hypercortisolism (SH) is a condition of cortisol excess in the absence of its classical signs and symptoms (i.e. striae rubrae, proximal myopathy, facial plethora, easy bruising) and it may be of both exogenous (i.e. iatrogenic) and endogenous origin (1-5).

The possible skeletal damage due to the low oral dose of glucocorticoids (GCs, i.e. <5 mg/die prednisone equivalents) is well known since many years (6-8) and it is still a matter of concern even in patients taking GCs at substitutive doses for adrenal insufficiency (8-14).

The prevalence of the endogenous form of SH is probably higher than previously suspected (3, 15), considering that a subtle cortisol excess is estimated to be present in the 5-30% of patients bearing an incidentally discovered adrenal adenoma (adrenal incidentaloma, AI) (16-19). Since an adrenal incidentaloma is estimated to be present in up to the 4-7% of the adults (20, 21), the prevalence of SH may be between 0.2% and 2.0% (1, 3). Less frequently, an endogenous SH is due to a slight adrenocorticotropic hormone (ACTH) excess (22, 23). Given its high prevalence, the possible chronic consequences of SH are being deeply investigated. Besides hypertension and metabolic syndrome, this condition has been suggested to be detrimental for the skeletal health. Indeed, SH may lead to an increased risk of vertebral fractures, partially explained by a reduction of bone mineral density (BMD) and possibly associated with a decreased bone quality (24).

Several aspects of the SH related skeletal damage are currently debated. Firstly, the role of the dual X-ray Absorptiometry (DXA) in predicting the risk of fractures is still unclear, since SH seems to impair bone microarchitecture rather than bone density (25-27). Secondly, the individual sensitivity to cortisol due to the different polymorphisms of the glucocorticoid receptor and to the different activity of 11beta-hydroxysteroid dehydrogenase enzymes may influence the skeletal effect of hypercortisolism (28, 29). Finally, an endogenous SH may be more frequent than expected in osteoporotic patients (30), in whom the vertebral fractures may be the presenting symptoms of an otherwise asymptomatic cortisol excess (31).

The present review will summarize the available data regarding the bone turnover, bone mineral density, bone quality and risk of fracture in patients with endogenous SH. In addition, the role of the individual glucocorticoid sensitivity in SH related bone damage and the problem of diagnosing and
managing the bone consequences of SH will be reviewed. Finally, the issue of suspecting and screening for SH patients with apparent primary osteoporosis will be addressed.
Bone turnover, bone mineral density, risk of fracture and bone quality in patients with endogenous SH

Bone Turnover

The available studies regarding the bone turnover, bone density, risk of fractures and bone quality in SH patients are summarized in Table 1. As far as bone turnover is concerned, several studies reported a reduction of bone formation as measured by osteocalcin in SH patients (32, 33, 34, 35, 36, 37). This finding is in keeping with the fact that the glucocorticoid excess inhibits the osteoblastic differentiation and activity and increases the osteoblastic apoptosis (38). However, other studies did not find a reduction of bone formation as evaluated by osteocalcin (39-41) and/or bone alkaline phosphatase (32, 36). These discordances are likely due to the small sample size of the available studies, to the low reliability of the bone formation markers (42) and to the difference in the prevalence of eugonadal and hypogonadal patients and in the criteria used to define SH among the different studies. Moreover, it should be noted that, particularly in the condition of glucocorticoid excess, osteocalcin and alkaline phosphatase are not good markers of formation since they are directly regulated by glucocorticoids, and are, thus, disproportionately reduced by glucocorticoids. However, it is well known from clinical and experimental studies that glucocorticoids impair osteoblastic function, either by bone marker or by histomorphometric studies (38), and, therefore, an impaired bone formation is likely to be present even in SH patients.

Data on bone resorption are more discordant, also depending on the marker assessed. The carboxyterminal telopeptide of type 1 collagen levels have been found to be enhanced (32, 37), normal (34, 40) or reduced (33), while the urinary deoxy-pyridinoline levels have been concordantly reported to be normal (34-36, 39, 40). Data regarding parathyroid hormone (PTH) levels in SH patients are scarce. Our data showed that AI female patients with SH had higher PTH levels than patients without SH (34, 35), and in one study PTH levels were inversely correlated with femoral BMD (34). This association between femoral bone mass and PTH was also reported in the studies by Hadjidakis and coauthors (41) and Osella and coauthors (48), even if in both studies in AI patients with SH the PTH levels were not increased. Again, the small sample size of the studies and the difference between the criteria used for defining SH may explain the discordant data on bone resorption and PTH levels in SH. In addition, the increased bone resorption, that was found in some studies (32, 37), could be even due to the post-menopausal status of some patients included in
the studies, as it is known that in the condition of estrogen deficiency the skeletal tissue is more sensitive to
the glucocorticoid excess (38).

In summary, in SH an uncoupling between bone apposition and resorption is present with the
osteoblastic activity being predominantly affected, as happens in the overt form of hypercortisolism.

**Bone mineral density**

As expected on the basis of the known detrimental effect of cortisol excess on trabecular bone (38),
most studies found a reduction of trabecular BMD measured at spine by DXA (26, 27, 34, 35, 37, 39, 40, 43-47) and/or quantitative computed tomography (35, 44) and by ultrasound at the proximal phalanges (43).

It must be observed that the studies that did not found a reduction of trabecular BMD in SH (36, 41, 47, 48)
have important limitations. In the study by Rossi and coauthors, BMD was measured only in patients <65
years (47). In the study by Osella and coauthors, the range of the Z-scores (numbers of SD from the mean
BMD of the age- and sex-matched control subjects) in the control group was extremely large and, in fact, 4
out of 5 post-menopausal SH women had reduced BMD (48). Finally, in the study by Hadjidakis and
coauthors a healthy control group was not included (41) and in the study of Francucci and coauthors only
two SH patients were studied (36).

Therefore, given the amount of data showing a reduction of trabecular BMD in AI patients and the important
pitfalls of all negative studies, in our opinion the association between SH and the reduction of trabecular
BMD should be considered well demonstrated.

On the contrary, data regarding cortical bone are more discordant. Indeed, several studies from our
group and from other Authors showed that in AI patients with SH, the BMD at femur was reduced (26, 27,
34, 37, 40, 41, 43, 45). However, in other reports a normal femoral BMD in SH patients was found (35, 39,
46). It must be observed that in the largest available study (26) and in the studies enrolling only post-
menopausal female patients or only eugonadal males the femoral BMD was consistently found to be reduced
(34, 42, 37, 45). Overall, even though the small sample size and the selection criteria of the population
studied might have influenced the results obtained, it is conceivable that in SH, as in overt cortisol excess
(38) the cortical bone might be relatively preserved.

Studies assessing the change of BMD over time in SH patients are scarce and somewhat conflicting.
While in a previous study an increased rate of bone loss was found in female patients with higher 24 hours
urinary free cortisol (UFC) levels (35), in two subsequent studies the BMD did not vary over time in SH patients (50, 51). These apparently discordant findings are probably due to the different disease activity of the SH patients studied. Indeed, the mean UFC levels of SH patients in the former study were slightly higher (i.e. 75.7 µg/24-hour, 209 nmol/24-hour) as compared with those of the subsequent studies (66.5 and 63.7 µg/24-hour, 183 and 176 nmol/24-hour, respectively).

In summary, SH probably affects trabecular bone at spine and possibly cortical bone at femur and an increased bone loss over time may be appreciated particularly in patients with higher cortisol levels.

Risk of fractures and bone quality

At variance with data on bone turnover and BMD, data regarding the risk of fracture are fully concordant and show that in SH patients the prevalence of vertebral fractures varies between 46.3% and 82.4%, and that it is higher than in control subjects (26, 27, 35, 44-46, 49-52). In addition, the available longitudinal studies showed that the 24-48% of SH patients may experience a new asymptomatic vertebral fracture over time (50-52), while AI patients with SH, who underwent the surgical removal of the adrenal mass, had a strong reduction of the probability of a new vertebral fracture (51).

We conducted a random effects meta-analysis using the 9 available studies assessing the prevalence of vertebral fracture in SH. The estimated prevalence of vertebral fractures among AI patients with SH is 63.6% (95%CI: 55.98-71.26%) in the presence of a certain grade of heterogeneity as suggested by the Q test for heterogeneity (19.09, p=0.014) and by the I^2=58% (Figure 1). The same analysis has been performed for assessing the prevalence of vertebral fractures in AI patients without SH (X studies, Figure 2) and in the control populations when available (X studies, Figure 3). These analyses suggest that the prevalence of vertebral fractures is increased even in patients apparently without SH (28%, 95%CI: 20-35%) than in controls (16%, 95%CI 5-28%, Cochran Q test Q=2.72, p=0.099). This is explained by the fact that some patients apparently without a clear biochemical picture of SH are, in fact, affected with a slight degree of hypercortisolism. Indeed, to date, the diagnosis of SH is still a challenge for clinicians, since, particularly in AI patients, cortisol secretion is highly fluctuant over time and is a continuum from completely normal to clearly increased levels and, therefore, the currently used parameters of cortisol secretion may be not enough sensitive for detecting a slight hypercortisolism in all affected individuals (1, 3).
The increased fracture risk in SH seems to be independent of gender and gonadal status. Indeed, in the largest cross-sectional study available so far, we found that even the premenopausal females and eugonadal males with SH have an increased prevalence of asymptomatic vertebral fractures (54.5% and 68.8%, respectively) as compared with healthy age- and gonadal status matched control subjects (26). In keeping, in a subsequent longitudinal study we demonstrated that in SH patients the risk of a new vertebral fracture is 12-fold increased regardless of age, gender, BMD and other possible confounders as compared with patients without SH (50). Apparently surprising, the degree of this risk in SH is similar to that reported in overt cortisol excess (24). However, SH is asymptomatic and, therefore, at the first diagnosis the duration of the hypercortisolism in SH patients has been probably longer than that in patients with a clinically overt cortisol excess. In keeping, in the study by Tauchmanovà and coauthors the disease duration was found to be inversely correlated to BMD measured by finger ultrasound (43).

Interestingly, in SH the degree of the BMD reduction is scarcely predictive of the fracture risk. This has been well demonstrated in several studies of our Group showing that the 40% of the eugonadal male AI subjects experienced a vertebral fracture in spite of a normal or only slightly reduced BMD (45) and that the presence of vertebral fracture at baseline and the occurrence of a new vertebral fracture during follow-up were independent of spinal BMD (26, 45, 50) and of other possible confounders (i.e. age and gender).

The reduced reliability of BMD in predicting the fracture risk in SH suggests that, as in patients with overt cortisol excess (38) even in subjects with a subclinical form of hypercortisolism a reduction of bone quality (i.e. bone microarchitecture), besides the decrease of bone density, is among the mechanisms underlying the increased fracture risk. However, bone quality can be assessed directly only by histomorphometric analysis of invasively obtained bone biopsy (53) or by micro–computed tomography systems (54). However, the idea of a reduced bone quality in SH was firstly suggested by two studies (26, 50) of our Group, evaluating in AI patients the spinal deformity index (SDI). The SDI is a semiquantitative method that integrates the number and the severity of vertebral fractures (55), that, in turn, have been suggested to be a surrogate index of bone microarchitecture (56). Therefore, the SDI may indirectly give information on bone quality. In the first study, we found that the SDI was higher in patients with SH than in those without SH and associated with the presence of SH regardless of age, body mass index, gender, spinal
BMD and gonadal status (26). In the subsequent study we demonstrated that the SDI worsened over time in AI patients with SH but not in those without SH (50).

More recently, the bone microarchitecture in AI patients has been indirectly studied by using the Trabecular Bone Score (TBS). This technique provides a gray-level texture measurement based on the use of experimental variograms of two-dimensional (2D) projection images easily obtainable during a DXA scan (57), and it has been demonstrated to be strongly correlated with bone microarchitecture, regardless of BMD (58). In a study on 102 AI patients, we showed that TBS was reduced in AI patients with SH as compared with AI patients without SH and controls, and that it was correlated with the number and severity of vertebral fractures and associated with the presence of vertebral fractures and with the degree of cortisol excess, after adjustment for potential confounding factors (27). Finally, in a subgroup of patients followed for 24 months, TBS was associated with the occurrence of a new vertebral fracture regardless of spinal BMD and other potential confounders.

In summary, these data show that in SH the risk of vertebral fracture is increased regardless of age, gender, gonadal status and BMD and that it is probably associated with a reduction of bone microarchitecture at least as indirectly evaluated by SDI and TBS. In keeping with the data regarding other possible consequences of SH, such as diabetes and hypertension (59), the recovery from SH seems to importantly reduce the vertebral fracture risk.

Pathogenesis and role of the individual glucocorticoid sensitivity in SH related bone damage.

The description of the cellular mechanisms underlying the bone damage in hypercortisolism is beyond the scope of this review and it has been recently reviewed elsewhere (60). It is well known that the pharmacological GC administration is rapidly followed by a transient increase of osteoclast number and bone resorption and by an osteoblast inactivation and decreased bone formation, that lead to a rapid bone loss and increased risk of fracture. Subsequently, the bone resorption decreases and the persistent inhibition of osteoblastic activity remains the main cause of the loss of bone density and quality and of the persistent increase in fracture risk (60). Given the long disease duration in patients with the endogenous form of hypercortisolism, and in particular in those with SH, the main mechanism underlying the skeletal damage is the reduction of the osteoblastic activity and of bone apposition, as indicated by the bone turnover data.
An important difference between the endogenous and the exogenous form of the glucocorticoid excess is that in the latter the effect of the glucocorticoid administration on bone tissue may be influenced by the skeletal impact of disorders themselves (e.g. rheumatoid arthritis and systemic lupus erythematosus) for whom the glucocorticoids are given (61).

Besides its direct negative effect on the bone cells, the cortisol excess indirectly affects the skeleton by inhibiting the hypothalamic-pituitary-gonadal axis activity and the growth hormone (GH) - insulin like growth factor I axis, as shown in patients affected with the clinically overt form of hypercortisolism (62, 63). It is unknown whether SH may lead to hypogonadotropic hypogonadism. At variance, some studies have evaluated the possible GH defect in SH patients (64-66). Two studies apparently did not report an altered GH reserve in AI patients with SH (64, 65). However, in the former the GH reserve was associated with the degree of cortisol secretion and the GH peak after GH releasing hormone tended to be lower in patients with SH than in those without (64) and in the latter study the four AI patients with ascertained SH showed a reduced GH reserve as compared with AI patients without SH (65). Finally, in a more recent study we found that GH reserve was decreased in SH patients in relation to cortisol hypersecretion, and that the GH secretion increases after the recovery from SH (66).

Another possible contributor to the skeletal damage in patients with ACTH-independent cortisol excess may be the possible reduced adrenal dehydroepiandrosterone-sulphate (DHEAS) secretion due to the blunted ACTH levels (67). Indeed, a correlation between DHEAS levels and BMD was described in patients with overt cortisol excess, accounting for a possible more severe bone involvement in patients with ACTH-independent hypercortisolism than in those with an ACTH-dependent form of cortisol excess, since in the latter patients the DHEAS secretion is normal or even high, while in the former it is usually blunted (68). In SH patients the role of DHEAS in contributing to the bone loss has been scarcely investigated. However, the few available data are not consistent with a possible main effect of DHEAS on bone in the condition of SH (37) and, in keeping, even in patients with overt clinical hypercortisolism the risk of vertebral fracture is independent of DHEAS levels (69, 70).

Recently, the issue of the role of the individual sensitivity to glucocorticoids in the skeletal tissue and in influencing the bone consequences of hypercortisolism has become a matter of debate. In Chinese (71) and in postmenopausal diabetic patients (72) the glucocorticoid receptor (GR) gene polymorphisms have
been suggested to possibly play a role in osteoporosis. In the general population, the BclI and the N363S polymorphism of the GR have been associated with low BMD (73, 74), while the ER22/23EK GR polymorphism is associated with a reduced sensitivity to glucocorticoids (75). In patients with clinically overt cortisol excess the role of the different GR polymorphisms has been investigated, but the results obtained have been conflicting. In a previous study patients carrying the BclI polymorphism of the GR showed reduced femoral BMD as compared with patients carrying the wild type GR (76), while in a subsequent study the GR gene variants seemed not to play a role (77). It is likely that in the presence of high cortisol levels, as in most patients with clinically overt cortisol excess, the role of the GR polymorphisms related glucocorticoid sensitivity may be limited. At variance, in the condition of a minimal cortisol excess, as in some individuals with diabetes (78), the GR polymorphism may influence the bone health.

Therefore, AI patients with and without SH might be an interesting model for studying the impact of the GR polymorphism in modulating the skeletal sensitivity to the glucocorticoid excess. However, in AI patients the few available data on this issue are not conclusive. In a previous study we found that in AI patients the contemporary presence of homozygous BclI and heterozygous N363S GR polymorphism was associated with fragility vertebral fracture (28). However, in a further study the association between the GR polymorphism and the BMD in AI patients was absent, but the possible differences in the presence of vertebral fractures were not assessed (79). It is possible to hypothesize that in these studies the small sample size could have influenced the results.

The other possible main determinant of the individual bone sensitivity to glucocorticoids is the 11beta-hydroxysteroid dehydrogenase type 1 (11betaHSD1) activity, that seems to be important for the bone health in the conditions of both normal and increased cortisol levels (80, 81). Indeed, in vitro studies showed that the inhibition of 11betaHSD1 improves osteoblast differentiation (82) and that it protects osteoblasts against the glucocorticoid induced dysfunction (83). In keeping with these data, the polymorphic variants of the 11betaHSD1 gene have been shown to be associated with BMD (84, 85) and fracture risk (86) in postmenopausal osteoporotic women without clinically apparent hypercortisolemia. The role of the 11beta-HSD1 in patients with cortisol excess has been scarcely investigated. Szappanos and coauthors showed that the 83,557insA variant of the gene coding 11betaHSD1 was associated with serum osteocalcin levels in patients with clinically overt glucocorticoid excess (87). A recent study showed that in AI patients
11betaHSD1 activity was not associated with the possible SH complications such as diabetes and hypertension (88), but its influence on BMD and on the fracture risk in patients with SH has never been studied.

**Management of the possible bone consequences in patients with SH**

*Diagnosis*

The first challenge in evaluating the possible bone consequences of SH is related to the fact that the presence of SH itself is often difficult to ascertain. The revision of the studies investigating the biochemical diagnosis of SH is beyond the aims of the present review and it has been recently published elsewhere (3-5, 59, 89). Looking at the Table 1, all studies addressing the topic of the bone involvement in SH, were performed on AI patients making an *a priori* classification in subjects with or without SH. Most studies used the presence of at least 2 altered parameters among the unsuppressed cortisol levels (i.e. >1.8 µg/dL, 50 nmol/L, or 3.0 µg/dL, 83 nmol/L, or 5.0 µg/dL, 138 nmol/L) after 1 mg dexamethasone overnight suppression test (1mgDST), increased UFC levels or reduced (<10 pg/dL or <5 pg/dL, <2.2 pmol/L or <1.1 pmol/L) ACTH levels. However, these criteria for diagnosing SH are probably not enough sensitive as suggested by the finding that in several studies the AI patients without SH, who have been surgically treated for the tumor size or growth, experienced the amelioration of some metabolic consequences of SH (47, 90-92). In keeping, as compared with pre-menopausal healthy control subjects the pre-menopausal AI patients without SH showed an increased prevalence of vertebral fragility fractures (49) and a reduced TBS (27). Because to date the diagnosis of SH is defined by using arbitrary cutoffs of indexes of cortisol secretion, it is possible to hypothesize that some patients classified as not having SH might have, in fact, a mild degree of cortisol hypersecretion.

In a study aimed to assess the diagnostic accuracy of cortisol levels after 1mgDST for predicting the risk of vertebral fracture in AI patients we showed that the confirmed cortisol level after 1mgDST ≥2 µg/dL (55 nmol/L), in the absence of possible interfering medications and/or diseases, was the best parameters for identifying patients at risk for fractures with a 80% sensitivity and a 68.8% specificity (52). The X-ray at spine should be repeated after 18 and 36 months since the first evaluation. In the absence of new vertebral fracture during a 36 months follow-up, a further X-ray evaluation is not useful. Otherwise, it
would increase the cancer risk and it is not justified by the available literature evidences. Unfortunately, no
data are available regarding the possible use of vertebral morphometry by DXA in SH patients.

The second difficulty in diagnosing the possible consequences of SH is related to the reduced
reliability of the BMD measurement in identifying the SH patients at risk for fractures. Indeed, although in
SH patients the spinal BMD is associated with the prevalent and incident vertebral fracture (3), it is probably
not enough sensitive for detecting individuals with asymptomatic vertebral fracture and the fracture risk is
independent of the BMD reduction (50). In this field the determination of TBS might be of some help. A
study on more than 100 AI patients with and without SH found that a TBS Z-score ≤1.500 and a spinal BMD
Z-score <0.00 showed a 79% specificity (sensitivity 50.8%) for predicting fractures, whereas a TBS Z-score
>1.5 plus a spinal BMD Z-score ≥0.0 had a 88.1% specificity (sensitivity 37.2%) for excluding fractures
(27).

Overall these data suggest that all AI patients with cortisol levels after 1mgDST ≥2 µg/dL (55
nmol/L) should undergo the determination of BMD by DXA and the assessment of the presence of vertebral
fractures by vertebral morphometry. In addition, since in SH the fracture risk is increased regardless of the
BMD reduction, all AI patients with possible or ascertained SH should be screened for the occurrence of new vertebral fractures during the follow-up. It is important to underline that the information regarding the bone status in SH patients may change the overall therapeutic approach in the individual AI patient with not yet
established SH. Indeed, in AI patients with possible SH the usefulness of surgery is debatable. However, if some possible SH consequences are present, the surgical approach has more probabilities to be successful
(93).

**Therapy**

Currently, only one study has been published on the effect on bone of the surgical treatment of AI
patients with SH. In this study on 55 patients with and without SH longitudinally followed for more than 24
months, surgery was associated with a 30% reduction of the fracture risk regardless of age, gender, follow-up
duration, cortisol after 1mgDST levels, spinal BMD, and presence of vertebral fracture at baseline (51).
Though not randomized, in this study the baseline characteristics of surgically treated and conservatively
managed subjects were comparable. Therefore, on the basis of available data surgery has to be considered in
SH patients at risk for fracture.
The only study evaluating the effect of a bone active drug on the risk of vertebral fracture suggested that weekly clodronate treatment prevents bone loss and vertebral fractures in women with SH (49). However, though randomized, the study had a follow-up of only 12 months. No patient treated with clodronate experienced a new vertebral fracture during the follow-up, while one subject among not treated patients had a new vertebral fracture (51).

Therefore, to date the effect of the medical therapy on the SH-related bone damage has still to be determined and specific guidelines for the management of patients with endogenous hypercortisolism are not available (94). As a consequence a single-case evaluation is often needed (95). In the absence of guidelines specifically designed for patients with endogenous glucocorticoid excess, it is reasonable that physicians could refer to the guidelines for the management of osteoporosis induced by exogenous glucocorticoid administrations (96, 97), which are generally based on the individual fracture risk profile (calculated by FRAX©) and dose of glucocorticoid used. However, it is not possible translating the corticosteroid dosages to the different degrees of endogenous hypercortisolism, and data on validation of FRAX stratification method in patients with SH are lacking. In addition, in patients with endogenous hypercortisolism the removal of the cause of the cortisol excess may determine the recovery of bone health. Consequently, it is unclear whether such recommendations may be adapted to patients with hypercortisolism, and particularly with SH (95).

However, some issues deriving from studies in patients with overt hypercortisolism may be useful in patients with SH. Firstly, all patients exposed to glucocorticoid excess should be treated with adequate supplementation of vitamin D and calcium (96, 97). This is particularly important in patients after the recovery from hypercortisolism (overt or subclinical), since the adequate vitamin D and calcium supplementation supports the rapid mineralization of the newly formed bone matrix (98). Secondly, although SH is generally diagnosed in women after menopause and rarely determines hypogonadism in males, the use of sex steroids for protecting bone in SH might be considered, at least looking at data in animal models (99). However, in patients with hypercortisolism, data regarding the protective role on bone of the sex steroids replacement therapy are lacking and the sex steroids administration might potentially worsen the cardiovascular risk. Thirdly, a reduced GH reserve has been described in patients with both overt cortisol excess (100) and SH (66) and the GH deficit has been associated with osteoporosis and fragility fractures.
In patients with SH the GH reserve seems to recover shortly after the normalization of cortisol levels (66), but if these patients are conservatively managed the GH deficit may persist for many years. Thus, theoretically, GH treatment could be an option in patients with SH induced osteoporosis (102). However, this therapy has potential drawbacks since both recombinant GH and cortisol excess may induce insulin resistance that may potentially result in glucose intolerance (103).

In the absence of widely accepted guidelines on the use of bone active drugs in endogenous cortisol excess, the approach proposed by the experts of the Altogether to Beat Cushing’s syndrome (ABC) Group for the management of patients with clinically overt hypercortisolism (95) might be largely applied also in SH patients, given that the pathogenesis and the risk of fracture in SH are comparable to those in clinically overt hypercortisolism (Figure 2). Therefore, premenopausal women or men aged <50 years, in the absence of fractures and of a BMD reduction, are at low risk and should be treated with only calcium and vitamin D if the recovery from hypercortisolism is rapidly expected. A bone-active treatment may be considered in patients who do not undergo surgery and who are older than 70 years of age or in post-menopausal women or men older than 50 years with a fragility fracture and/or a FRAX© calculated 10-year risk for fractures ≥20% and/or with BMD T-score < -1.5. In addition, a bone-active drug should be considered in both low risk and not surgically treated patients and surgically treated patients in the presence of a fragility fracture and/or the BMD decrease during the follow-up.

The ideal drug in the condition of SH should be able to counteract the negative skeletal effects of glucocorticoids, without impairing the recovery of bone remodeling after resolution of the cortisol excess. It is known that, bisphosphonates, Denosumab and Teriparatide are effective in counteracting the negative effects of glucocorticoids on bone (96, 97, 104). The available data in patients with the endogenous hypercortisolism suggest that alendronate and clodronate have positive effects on BMD (49, 105), but in this clinical context their anti-fracture effect is unknown. At diagnosis the patients with endogenous cortisol excess present with a reduced bone apposition and normal or only slightly increased bone resorption. Therefore, the bisphosphonates, by further suppressing bone turnover, in theory could favor the occurrence of rare undesired effects, such as atypical subtrochanteric fractures and osteonecrosis of the jaw, which are, moreover, more frequent in the condition of glucocorticoid excess (106). In addition, the bisphosphonates have a long-term antiresorptive effect, which may negatively influence the recovery of bone apposition after
the correction of hypercortisolism. However, in SH patients without a very low bone resorption the bisphosphonates could re-establish the balance between formation and resorption and could be successfully used. At variance, Denosumab, given its rapid and transient antiresorptive effect, could be used for a short-term treatment in patients waiting for the definitive cure of the endogenous hypercortisolism, even though it must be underlined that, currently, this would be an off-label use of the drug (107). However, since the inhibition of bone apposition is the main pathophysiological mechanism leading to bone loss in patients with endogenous hypercortisolism (103), Teriparatide should be considered the best drug to treat skeletal fragility in patients with hypercortisolism (106, 108). However, the effectiveness and long-term safety of teriparatide in patients with endogenous hypercortisolism have been scarcely investigated, even in consideration that the adrenal cortisol secretion may be increased by the use of this drug (109). In addition, it is important that the potential malignant nature of the adrenal tumor is definitely excluded before the therapy with teriparatide is initiated. Finally, although data on animal models suggest the possible use of strontium ranelate in the glucocorticoid-induced osteoporosis (110), data on the efficacy of strontium ranelate in patients with hypercortisolism are not available. However, given that this drug is associated with an increased risk of cardiovascular events, it is probably not safe to suggest this drug in SH patients, who themselves already are at increased risk of cardiovascular disease.

In summary, in patients at low risk for fracture and in whom the cortisol excess is expected to be rapidly corrected, an anti-osteoporotic drug is not needed and only calcium and vitamin D should be given. In patients conservatively treated at high risk for fracture and in patients in whom the surgical treatment has to be postponed an anti-osteoporotic drug in addition to calcium and vitamin D should be started. In these patients Teriparatide could be the drug of choice, even though, due to its high cost, it should be reserved for patients at very high risk of clinical fracture (i.e. with prevalent asymptomatic vertebral fractures).

The need of a bone-active drug after the recovery from endogenous hypercortisolism has been advocated on the basis of data showing that the risk of fractures remains high within 2 years from surgery (111). However, it must be considered that in the past years the daily dose of the steroid replacement were definitely higher than that currently used (112), and, therefore, these data have been probably biased by an overtreatment of the post-surgical hypoadrenalism. In addition, the usefulness of bisphosphonates after the recovery from the endogenous hypercortisolism is questioned (113). Therefore, after the recovery from SH a
bone-active drug should be initiated only in the presence of a BMD decrease and/or if a fragility fracture occurs during the follow-up (Figure 2).

Prevalence of the endogenous form of SH in patients with apparent primary osteoporosis

It is known since many years that a condition of otherwise asymptomatic hypercortisolism may present itself with the occurrence of a fragility vertebral fracture and/or unexplained osteoporosis as unique sign (31, 114, 115). In addition, given the recent evidences showing that SH is definitely more frequent than the clinically overt cortisol excess and that SH is associated with an increased risk of osteoporosis and fragility fractures, several studies have been designed for assessing the prevalence of an otherwise asymptomatic SH in patients with osteoporosis (30, 116-119).

The Table 2 summarized the studies specifically designed for assessing the prevalence of SH among patients with apparent primary osteoporosis. As shown, the SH prevalence in the osteoporotic population varies from 0.6% to 3.8% and from 1.9% to 17.6% in patients with osteoporosis and vertebral fractures. The differences in the SH prevalence are probably due to the different screening test, setting of the studies and type and sample size of the population studied. The only study that failed to find an increased prevalence of SH among osteoporotic patients screened a sample of osteoporotic women by determining the UFC levels (117), that are notoriously not enough sensitive to detect SH (3, 31, 59). With the exception of that study, in all other studies SH has been screened by using cortisol levels after 1mgDST with a cut-off of 1.8-2.0 µg/dL (50-55 nmol/L) and, subsequently, SH has been confirmed by the commonly used additional second-line tests. Using this protocol the SH prevalence among patients referred to an outpatient clinic for osteoporosis was between 1.3% and 3.8%, but in two studies reached almost the 11% and 18% if the screening was confined to patients with fragility fracture. However, it must be considered that these latter studies have been conducted in tertiary care center for osteoporosis and metabolic bone diseases (30, 119) and that SH was searched for after all possible other causes of secondary osteoporosis had been excluded. Finally, the different sample size of the available studies (between 80 and 600 patients) may have influenced these results. Notwithstanding these limitations, the data on the SH prevalence in osteoporosis are in keeping with data on the SH prevalence in other populations that might be at increased risk of SH, such as in patients with
diabetes or hypertension. Indeed the presence of SH was described in the 2.1-5.5% of diabetic patients (120-121) and in up to the 8% of the hypertensive patients (122-123).

Overall, it is possible to hypothesize that the 1-4% of patients with apparently primary osteoporosis has, in fact a slight cortisol excess. Given that SH is a potentially removable condition, the possibility of a SH screening in the populations at risk is a matter of debate (124, 125). In our opinion, the current evidences do not consent to extend the SH screening to all patients with osteoporosis and/or fragility fracture as first-line test. The table 3 summarizes the indications for the SH screening in patients with osteoporosis and/or fragility fractures as suggested by some leading experts. As shown, it is reasonable to screen for SH all subjects with low BMD as compared to age- and weight-matched controls and/or if BMD declines more rapidly than expected and/or if it fails to respond to appropriate therapy and/or in the presence of fragility fractures in eugonadal persons (126, 127). Importantly, the precocious diagnosis of SH may help to prevent also the extra-skeletal consequences of this condition of subtle hypercortisolism (128).

Conclusions

The literature evidence clearly shows that AI patients with SH are at high risk of osteoporosis and fragility fractures and that the presence of SH should be always suspected in patients with established and otherwise unexplainable osteoporosis. These data are important from a clinical point of view, since in the management of patients with SH the possible bone damage should be taken into account and in the management of patients with established osteoporosis the presence of SH should be suspected.

From a pathophysiological point of view, the condition of SH in AI patients may represent a pure form of slight cortisol excess. Differently from the exogenous hypercortisolism, in the endogenous hypercortisolism the confounding effect of the diseases for which the glucocorticoids are given, is absent (129). Therefore, studying the bone involvement in AI patients with SH may consent to assess some important aspects of the pure effect of glucocorticoid on bone, such as, for example, the role of the individual sensitivity to glucocorticoid excess.

This field of research will probably be important not only in the condition of glucocorticoid excess but in general in several diseases characterized by enhanced, though still normal, cortisol secretion such as in diabetes (72, 78) but also in some crucial phases of the life for bones. Indeed, recent data show that in
healthy children, higher glucocorticoid secretion in the physiological range is associated with lower bone strength at the proximal radius (130) and that in the early post-menopausal period several parameters of cortisol secretion are associated with BMD (131).

Finally, if the importance of the glucocorticoid secretion and sensitivity for bone health will be confirmed in future studies, it will be possible to personalize the clinical work-up on the basis of the individual risk of fracture and to try to counteract in some patients the adverse effect of the relatively increased cortisol secretion with the 11betaHSD1 inhibitors. Indeed, some studies showed that the inhibition of the 11betaHSD1 may protect bone against the deleterious effects of glucocorticoids (82, 83, 132).

In conclusion, physicians should keep in mind that SH, although asymptomatic, is not a harmless condition and the research should face the problem of the SH diagnosis and medical therapy.
The X-ray at spine should be repeated after 18 and 36 months since the first evaluation. In the absence of new vertebral fracture during a 36 months follow-up, a further X-ray evaluation is not justified by the available data and patients should be monitored only with dual X-ray Absorptiometry.

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**Figure Legend**

**Figure 1**
Random effects meta-analysis using the 9 published studies assessing the prevalence of vertebral fracture in patients with adrenal incidentalomas and subclinical hypercortisolism. The estimated prevalence of vertebral fractures is 63.6% (95%CI: 55.98%-71.26%) in the presence of a certain grade of heterogeneity as suggested by the Q test for heterogeneity (19.1, p=0.014) and by the $I^2=58$

**Figure 2**
Random effects meta-analysis using the 6 published studies assessing the prevalence of vertebral fracture in patients with adrenal incidentalomas and without subclinical hypercortisolism. The estimated prevalence of vertebral fractures is 28% (95%CI: 20-35%) in the presence of a certain grade of heterogeneity as suggested by the Q test for heterogeneity (16.7, p=0.005)

**Figure 3**
Random effects meta-analysis using the 4 published studies assessing the prevalence of vertebral fracture in the control subjects included in the studies assessing the prevalent vertebral fractures in patients with adrenal
incidentalomas. The estimated prevalence of vertebral fractures is 16% (95%CI: 5-28%) in the presence of a
certain grade of heterogeneity as suggested by the Q test for heterogeneity (65.3, p<0.0001).

Figure 4

Proposed flow-chart for the diagnosis, therapy and follow-up of the bone consequences of adrenal subclinical
hypercortisolism

AI: adrenal incidentalomas. 1mgDST: 1 mg dexamethasone overnight suppression test

1in the absence of possible interfering medications e/o diseases.

2if surgery is not practicable shortly, in patients with fractures and/or reduced bone mineral density bone-
active drugs, and in particular Teriparatide, may be considered.
**Table 1:** Summary of the available studies assessing bone turnover and/or bone mineral density and/or prevalence (and/or incidence) of fragility fractures in patients with unilateral adrenal incidentalomas and subclinical hypercortisolism.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>n. F/M</th>
<th>Eug/Hyp</th>
<th>SH (n.)</th>
<th>Bone Apposition</th>
<th>Bone Resorption</th>
<th>LS BMD</th>
<th>Fem BMD</th>
<th>Prev VFx</th>
<th>Main parameters for SH diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osella, 1997 (ref #32)</td>
<td>Cross-sect. Controlled</td>
<td>22/13/9</td>
<td>12/10</td>
<td>n.a.</td>
<td>OC: L</td>
<td>CTX: H</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>4 and 6 pts had 1mgDST &gt;5 and &gt;3 µg/dL, respectively</td>
</tr>
<tr>
<td>Sartorio, 1998 (ref #33)</td>
<td>Cross-sect. Controlled</td>
<td>41/27/14</td>
<td>n.a.</td>
<td>6</td>
<td>OC: L</td>
<td>CTX: L</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>1mgDST &gt;5 µg/dL in all SH pts; low ACTH and/or high UFC in 4 SH pts.</td>
</tr>
<tr>
<td>Torlontano, 1999 (ref #34)</td>
<td>Cross-sect. Controlled</td>
<td>32/32/0</td>
<td>8/24</td>
<td>8</td>
<td>OC: L</td>
<td>CTX: N</td>
<td>L</td>
<td>L</td>
<td>n.a.</td>
<td>SH if UFC &gt; 70 µg/24h</td>
</tr>
<tr>
<td>Rossi, 2000 (ref #47)</td>
<td>Cross-sect.</td>
<td>50/29/21</td>
<td>n.a.</td>
<td>12</td>
<td>n.a.</td>
<td>n.a.</td>
<td>N&lt;sup&gt;1&lt;/sup&gt;</td>
<td>N&lt;sup&gt;1&lt;/sup&gt;</td>
<td>n.a.</td>
<td>≥2 out: 2mgDST &gt;3 µg/dL, low ACTH, high UFC, high F rhythm.</td>
</tr>
<tr>
<td>Osella, 2001 (ref #48)</td>
<td>Cross-sect. Controlled</td>
<td>27/18/9</td>
<td>n.a.</td>
<td>8</td>
<td>n.a.</td>
<td>n.a.</td>
<td>N</td>
<td>N</td>
<td>n.a.</td>
<td>≥2 out: 2mgDST &gt;3 µg/dL, low ACTH, high UFC, high F rhythm.</td>
</tr>
<tr>
<td>Chiodini, 2001 (ref #35)</td>
<td>Prospective Observational</td>
<td>24/24/0</td>
<td>8/16</td>
<td>7</td>
<td>OC: L</td>
<td>D-Pyr/Cr: N</td>
<td>L&lt;sup&gt;2&lt;/sup&gt;</td>
<td>N</td>
<td>n.a.</td>
<td>≥2 out: 1mgDST &gt;3 µg/dL, low ACTH, high UFC.</td>
</tr>
<tr>
<td>Tauchmanová, 2001 (ref #43)</td>
<td>Cross-sect. Controlled</td>
<td>19/11/8</td>
<td>19/0</td>
<td>19</td>
<td>n.a.</td>
<td>n.a.</td>
<td>L</td>
<td>L</td>
<td>n.a.</td>
<td>≥2 out: 2mgDST &gt;3 µg/dL, low ACTH, high UFC, high F rhythm.</td>
</tr>
<tr>
<td>Francucci, 2002 (ref #36)</td>
<td>Cross-sect. Controlled</td>
<td>23/23/0</td>
<td>5/18</td>
<td>2</td>
<td>OC: L</td>
<td>D-Pyr/Cr: N</td>
<td>N</td>
<td>N</td>
<td>n.a.</td>
<td>≥2 out: 2mgDST &gt;3 µg/dL, low ACTH, high UFC, high F rhythm.</td>
</tr>
<tr>
<td>Chiodini, 2002 (ref #39)</td>
<td>Cross-sect. Controlled</td>
<td>38/0/38</td>
<td>38/0</td>
<td>13</td>
<td>OC: N</td>
<td>D-Pyr/Cr: N</td>
<td>L</td>
<td>N</td>
<td>n.a.</td>
<td>≥2 out: 1mgDST &gt;3 µg/dL, low ACTH, high UFC.</td>
</tr>
<tr>
<td>Bardet, 2002 (ref #40)</td>
<td>Cross-sect.</td>
<td>35/22/13</td>
<td>13/22</td>
<td>18</td>
<td>OC: N</td>
<td>CTX: N</td>
<td>L</td>
<td>L</td>
<td>n.a.</td>
<td>1mgDST &gt;1.8 µg/dL and unilateral 131I Cholesterol uptake</td>
</tr>
<tr>
<td>Hadjidakis, 2003 (ref #41)</td>
<td>Cross-sect.</td>
<td>42/42/0</td>
<td>0/42</td>
<td>18</td>
<td>OC: N</td>
<td>n.a.</td>
<td>N</td>
<td>L</td>
<td>n.a.</td>
<td>2mgDST &gt;2.5 µg/dL</td>
</tr>
<tr>
<td>Chiodini, 2004 (ref #44)</td>
<td>Cross-sect. Controlled</td>
<td>70/70/0</td>
<td>21/49</td>
<td>21</td>
<td>n.a.</td>
<td>n.a.</td>
<td>L&lt;sup&gt;3&lt;/sup&gt;</td>
<td>n.a.</td>
<td>66.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≥2 out: 1mgDST &gt;3 µg/dL, low ACTH, high UFC.</td>
</tr>
<tr>
<td>Tauchmanová, 2007 (ref #37)</td>
<td>Cross-sect. Controlled</td>
<td>35/35/0</td>
<td>20/15</td>
<td>35</td>
<td>OC: L</td>
<td>CTX: H</td>
<td>L</td>
<td>L</td>
<td>57.0&lt;sup&gt;5&lt;/sup&gt;</td>
<td>1mgDST &gt;3.0 µg/dL</td>
</tr>
<tr>
<td>Chiodini, 2009 (ref #26)</td>
<td>Cross-sect. Controlled</td>
<td>287/176/111</td>
<td>142/145</td>
<td>85</td>
<td>n.a.</td>
<td>n.a.</td>
<td>L</td>
<td>L</td>
<td>70.6&lt;sup&gt;6&lt;/sup&gt;</td>
<td>≥2 out: 1mgDST &gt;3 µg/dL, low ACTH, high UFC.</td>
</tr>
<tr>
<td>Chiodini, 2009 (ref #45)</td>
<td>Cross-sect. Controlled</td>
<td>90/0/90</td>
<td>90/0</td>
<td>22</td>
<td>n.a.</td>
<td>n.a.</td>
<td>L</td>
<td>L</td>
<td>72.7</td>
<td>≥2 out: 1mgDST &gt;3 µg/dL, low ACTH, high UFC.</td>
</tr>
<tr>
<td>Tauchmanová, 2009 (ref #49)</td>
<td>Prospective Intervention</td>
<td>46/46/0</td>
<td>46/0</td>
<td>46</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>63.0&lt;sup&gt;7&lt;/sup&gt;</td>
<td>1mgDST &gt;3.0 µg/dL</td>
</tr>
<tr>
<td>Study</td>
<td>Study Design</td>
<td>n.</td>
<td>1mgDST</td>
<td>ACTH</td>
<td>UFC</td>
<td>F/M</td>
<td>Eug/Hyp</td>
<td>SH</td>
<td>LS</td>
<td>Fem</td>
</tr>
<tr>
<td>-------------------</td>
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<td>-----</td>
<td>-------</td>
<td>----</td>
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<td>-----</td>
</tr>
<tr>
<td>Morelli, 2011</td>
<td>Prospective</td>
<td>103</td>
<td>n.a.</td>
<td>27</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>55.6</td>
</tr>
<tr>
<td>Eller-Vainicher, 2012</td>
<td>Cross-sect. Controlled</td>
<td>102</td>
<td>63/39</td>
<td>n.a.</td>
<td>34</td>
<td>n.a.</td>
<td>L</td>
<td>L</td>
<td>82.4</td>
<td>≥2 out: 1mgDST &gt;3 µg/dL, low ACTH, high UFC.</td>
</tr>
<tr>
<td>Morelli, 2013</td>
<td>Cross-sect.</td>
<td>175</td>
<td>116/59</td>
<td>75/100</td>
<td>41</td>
<td>n.a.</td>
<td>L</td>
<td>N</td>
<td>46.3</td>
<td>≥2 out: 1mgDST &gt;3 µg/dL, low ACTH, high UFC.</td>
</tr>
<tr>
<td>Salcuni, 2015</td>
<td>Prospective</td>
<td>55</td>
<td>32/23</td>
<td>55/0</td>
<td>55</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>54.5</td>
<td>≥2 out: 1mgDST &gt;3 µg/dL, low ACTH, high UFC.</td>
</tr>
<tr>
<td>Morelli, 2015</td>
<td>Cross-sect.</td>
<td>444</td>
<td>272/172</td>
<td>n.a.</td>
<td>216</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>65.7</td>
<td>1mgDST ≥2 µg/dL.</td>
</tr>
</tbody>
</table>


1 Only 1 measurement site per person (spine in patients <65 years of age, femoral neck in patients ≥65 years of age).
2 Measured also by both quantitative computed tomography; increased rate of bone loss at spine but not at femur.
3 Measured only by quantitative computed tomography; the statistical significance was reached only in the post-menopausal subjects group.
4 78.6% in hypogonadal and 42.9% in eugonadal patients.
5 80% in hypogonadal and 40% in eugonadal patients; SH patients showed also increased prevalence of clinical fractures (11.4%), multiple fractures (31%) and rib and/or foot fractures (8.5%). 76.2% in post-menopausal female patients, 54.5% in premenopausal female patients and 68.8% in eugonadal male patients.
6 In patients conservatively treated: 61% and 65% at baseline and at the end of 12 months follow-up, respectively; in patients treated with clodronate: 65% both at baseline and at the end of follow-up. No healthy control group was included. 8 81.4% after 2 years of follow-up. No healthy control group was included.
9 Patients with SH had lower Trabecular Bone Score as compared with patients without SH and control subjects. Patients without SH had lower Trabecular Bone Score than control subjects. 10 In patients conservatively treated: 65.2% and 91.3% at baseline and at the end of follow-up, respectively; in patients surgically treated: 46.9% both at baseline and at the end of follow-up. No healthy control group was included.
11 The cut-off of cortisol after 1mgDST for was determined by receiver operating characteristic (ROC) curve; 30 out of 126 (23.8%) patients experienced a new VFx during a ≥24 months follow-up. No healthy control group was included.
Table 2: Summary of the available studies investigating the prevalence of subclinical hypercortisolism (SH) in patients with apparently primary osteoporosis

<table>
<thead>
<tr>
<th>Study</th>
<th>n. (F/M)</th>
<th>Population</th>
<th>Screening test</th>
<th>Cut-off</th>
<th>Overall</th>
<th>Prevalence (%)</th>
<th>In patients with low BMD</th>
<th>In patients with fragility fracture</th>
<th>In patients with low BMD and/or fragility fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kann, 2001 (ref #32)</td>
<td>78 (78/0)</td>
<td>Osteoporosis and fragility fracture</td>
<td>3mgDST</td>
<td>2.0 µg/dL</td>
<td>3.8</td>
<td>n.a.</td>
<td>3.8 (3/78)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Tannebaum, 2002 (ref #117)</td>
<td>173 (173/0)</td>
<td>Osteoporosis</td>
<td>UFC</td>
<td>n.a.</td>
<td>0.6</td>
<td>0.6 (1/173)</td>
<td>0.6 (1/173)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Chiodini, 2007 (ref #30)</td>
<td>219 (200/19)</td>
<td>Normal BMD (n=72), osteopenia or osteoporosis (n=147)</td>
<td>1mgDST</td>
<td>1.8 µg/dL</td>
<td>3.2</td>
<td>9.9 (7/71)</td>
<td>10.8 (7/65)</td>
<td>n.a.</td>
<td>4.8 (7/147)</td>
</tr>
<tr>
<td>Eller-Vainicher, 2013 (ref #118)</td>
<td>602 (563/39)</td>
<td>Osteoporosis and/or fragility fracture</td>
<td>1mgDST</td>
<td>1.8 µg/dL</td>
<td>1.3</td>
<td>1.7 (7/412)</td>
<td>1.9 (7/361)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Lasco, 2014 (ref #119)</td>
<td>50 (50/0)</td>
<td>n.a.</td>
<td>1mgDST</td>
<td>1.8 µg/dL</td>
<td>1.5</td>
<td>n.a.</td>
<td>17.6 (3/50)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

SI conversion factors: serum cortisol 27.56, urinary cortisol 2.756, ACTH 0.22. In all studies subjects with specific signs and/or symptoms of cortisol excess were excluded.

n.: number of patients included; F/M: females/males; BMD: bone mineral density as measured by Dual-X ray Absorptiometry; n.a.: not available data; 1mgDST: cortisol after 1mg dexamethasone overnight suppression test; 2mgDST: cortisol after 2 mg 2 days dexamethasone suppression test; 3mgDST: cortisol after 3mg dexamethasone overnight suppression test. UFC: 24-hour urinary free cortisol. Normal BMD is defined in the presence of a T-score > -1.0. Osteopenia and osteoporosis are diagnosed in the presence of BMD T-score between -1.5 and -2.5 and < -2.5, respectively.

1In 4 patients the presence of an ACTH-dependent SH was confirmed by performing an high dose dexamethasone suppression test over 5 days and a CRH stimulation test; an adrenal endosonography showed in 3 patients a micro- and macronodular hyperplasia.

2Hypercortisolism was diagnosed based on elevated UFC levels and subsequent not specified abnormal dexamethasone suppression testing; 4 patients had increased UFC levels; fragility fractures have been not assessed.SH

3In patients with 1mgDST >1.8 µg/dL the diagnosis was confirmed in the presence of midnight serum cortisol <7.5 µg/dL and/or UFC >60.0 µg/24-hour); eventually 6 patients had an ACTH-independent SH and an adrenal adenoma and 1 patient had an ACTH-dependent SCS and an ACTH-secreting pituitary adenoma.

4In subjects with cortisol after 1mg DST >1.8 µg/dL, SH was diagnosed in the presence of cortisol after 2mgDST >1.8 µg/dL and/or elevated UFC levels.
**Table 3**: Indication for the screening of subclinical hypercortisolism (SH) in patients with apparently primary osteoporosis.

<table>
<thead>
<tr>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low BMD as compared to age- and weight-matched controls (Z-score &lt; −2.0)</td>
</tr>
<tr>
<td>BMD declines more rapidly than expected</td>
</tr>
<tr>
<td>BMD fails to respond to appropriate therapy</td>
</tr>
<tr>
<td>Presence of a fragility fracture in eugonadal males</td>
</tr>
<tr>
<td>Presence of a fragility fractures in pre-menopausal females</td>
</tr>
</tbody>
</table>

BMD: bone mineral density. Z-score: number of standard deviations above or below what's normally expected for age, sex and ethnic or racial origin.
<table>
<thead>
<tr>
<th>Study (year of publication)</th>
<th>n.events/total</th>
<th>Prevalence [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiodini, JCEM 2004</td>
<td>14/21</td>
<td>0.67 [0.47, 0.87]</td>
</tr>
<tr>
<td>Tauchmanova, JJE 2007</td>
<td>20/35</td>
<td>0.57 [0.41, 0.74]</td>
</tr>
<tr>
<td>Chiodini, Clin Endo 2009</td>
<td>10/22</td>
<td>0.73 [0.54, 0.91]</td>
</tr>
<tr>
<td>Tauchmanova, JJE 2009</td>
<td>29/46</td>
<td>0.83 [0.49, 0.77]</td>
</tr>
<tr>
<td>Chiodini, JCEM 2009</td>
<td>60/85</td>
<td>0.71 [0.61, 0.80]</td>
</tr>
<tr>
<td>Morelli, JBMR 2011</td>
<td>15/27</td>
<td>0.56 [0.37, 0.74]</td>
</tr>
<tr>
<td>Eiler-Vainscher, JBMR 2012</td>
<td>28/34</td>
<td>0.82 [0.70, 0.95]</td>
</tr>
<tr>
<td>Morelli, EJE 2013</td>
<td>19/41</td>
<td>0.46 [0.31, 0.62]</td>
</tr>
<tr>
<td>Salcuri, EJE 2015</td>
<td>30/55</td>
<td>0.55 [0.41, 0.68]</td>
</tr>
</tbody>
</table>

Q test for heterogeneity=18.09, p=0.014
Random Effects Meta-Analysis 231/388 0.64 [0.56, 0.71]
Chodini, JCEM 2004 16/49 0.33 [0.20, 0.46]
Chodini, Clin Endo 2009 12/66 0.18 [0.09, 0.27]
Chodini, JCEM 2009 44/202 0.22 [0.16, 0.27]
Morelli, JBMR 2011 22/76 0.29 [0.19, 0.39]
Eler-Vainicher, JBMR 2012 31/68 0.46 [0.34, 0.57]
Morelli, EJE 2013 33/134 0.25 [0.17, 0.32]

Q test for heterogeneity = 10.71, p = 0.0051
Random Effects Meta-Analysis 158/595 0.28 [0.20, 0.35]
<table>
<thead>
<tr>
<th>Study (year of publication)</th>
<th>n.events/total</th>
<th>Prevalence [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiodini, JCEM 2004</td>
<td>23/84</td>
<td>0.27 [0.18, 0.37]</td>
</tr>
<tr>
<td>Tauchmanova, EJE 2007</td>
<td>1/71</td>
<td>0.00 [0.00, 0.04]</td>
</tr>
<tr>
<td>Chiodini, Clin Endo 2009</td>
<td>15/90</td>
<td>0.17 [0.09, 0.24]</td>
</tr>
<tr>
<td>Chiodini, JCEM 2009</td>
<td>43/194</td>
<td>0.22 [0.16, 0.28]</td>
</tr>
</tbody>
</table>

Q test for heterogeneity: 65.30, p < 0.0001

Random Effects Meta-Analysis

Prevalence

Prevalence

0.00  0.10  0.20  0.30  0.40  0.50  0.60
Figure 4

Proposed flow-chart for the diagnosis, therapy and follow-up of the bone consequences of adrenal subclinical hypercortisolism

AI: adrenal incidentalomas. 1mgDST: 1 mg dexamethasone overnight suppression test in the absence of possible interfering medications e/o diseases.

If surgery is not practicable shortly, in patients with fractures and/or reduced bone mineral density bone-active drugs, and in particular Teriparatide, may be considered.

254x190mm (300 x 300 DPI)