Progression of vertebral fractures in patients with adrenocortical carcinoma undergoing mitotane therapy

Progression of vertebral fractures in patients with adrenocortical carcinoma undergoing mitotane therapy

Deborah Cosentini MD[1], Salvatore Grisanti MD,PhD[1], Julien Hadoux MD, PhD[2], Rossella Libè, MD[3], Michele Frigerio MD[4], Marta Laganà MD[1], Frederic Deschamps MD[5], Manuel Zamparini MD[1], Livia Lamartina MD, PhD[2], Rebecca Pedersini MD[1], Clara Valsecchi MD[4], Roberto Maroldi MD[4], Abir Al Ghuzlan MD[2], Massimo Terzolo MD[6], Roberto Gasparotti MD[7], Eric Baudin MD, PhD[2], Alfredo Berruti MD[1]

[1] Medical Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia. ASST Spedali Civili, Brescia, Italy
[2] Department of Nuclear Medicine and Endocrine Oncology, and Interventional Medicine, Institute Gustave Roussy, Villejuif, France
[3] Department of Endocrinology, Cochin Hospital, Assistance Publique Hôpitaux de Paris, Paris, France
[4] Radiology Unit, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia. ASST Spedali Civili, Brescia, Italy
[5] Department of Interventional Radiology, Institute Gustave Roussy, Villejuif, France
[6] Internal Medicine, Department of Clinical and Biological Sciences, San Luigi Hospital, University of Turin, Orbassano, Italy
[7] Neuroradiology Unit, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia. ASST Spedali Civili Hospital, Brescia, Italy

Short title

Vertebral fractures in patients with adrenocortical carcinoma.

Key words

Adrenal tumor, bone health, mitotane, steroid supplementation, vertebral fractures

Corresponding author

Professor Alfredo Berruti
Oncologia Medica
ASST-Spedali Civili
Piazzale Spedali Civili 1
25123 Brescia
Italy
Email: alfedo.berruti@gmail.com

Funding

This work was supported in part by Fondazione Beretta, Brescia, Italy.

Disclosures

AB personal fees from Novartis, AAA, Janssen, Ipsen, Astellas, Amgen and research funds to the Institution from Astellas, Sanofi, Janssen, outside the submitted work. HJ personal fees from Pfizer, AAA, outside the submitted work. LL personal fees from EISAI, Bayer, outside the submitted work. MT grants from HRA RARE DISEASES, personal fees from HRA RARE DISEASES, outside the submitted work. DC travel grant from Fondazione Beretta. The other authors have nothing to disclose.

Abstract

Context: patients with adrenocortical carcinoma (ACC) are frequently on mitotane therapy for a long-time period. The drug exerts an adrenolytic activity requiring glucocorticoid supplementation, which can be potentially detrimental for bone.

Objectives: to explore whether mitotane plus/minus chemotherapy is associated with an increased proportion of morphometric vertebral fractures (VFs) in ACC patients. Secondary objectives were: proportion of patients with VF progression, or worsening of the spinal deformity index (SDI) during mitotane therapy; predictive factors of VF progression and prognostic role of VF progression.

Design and setting: multicenter, retrospective cohort study of patients with ACC who received mitotane alone or in association to chemotherapy, recruited from January 2010 to January 2020 in two reference centers in Italy and France.

Results: a significant increase in the frequency of VFs before and after mitotane therapy was seen both in Italian (28.3% vs 47.8%, p: 0.04) and French (17.8% vs 35.6%, p 0.04) series. VF progression was observed in 39.1%, and 28.9% of patients, respectively. Baseline VFs and increased patient body mass index, but not the dose of cortisol supplementation, showed an independent association with VF progression at multivariate analysis. Among the 72 advanced ACC patients, progression of VFs was associated with a poorer survival.

Conclusions: the administration of mitotane plus/minus chemotherapy in ACC patients impairs bone health independently from cortisol supplementation. Appropriate preventive measures to decrease the fracture risk should be implemented in these patients.

Introduction

Adrenocortical carcinoma (ACC) is a rare and aggressive malignant disease with an incidence of 0.7-2 new cases per million populations per year (1). The majority of ACC is functioning at presentation and Cushing syndrome is the most frequent clinical manifestation (2).

Radical surgery represents the mainstay of treatment of localized tumors (1) and it is the only treatment strategy that is able to offer a chance of cure to the patients. Despite radical resection, however, most patients are destined to relapse, often within the first 2 years. Based on the results of a retrospective cross-sectional study (3,4), whose results have been confirmed by other retrospective studies (1), adjuvant mitotane therapy for at least 2 years is currently prescribed and recommended by available guidelines (1,5). Single agent mitotane or the combination of mitotane plus chemotherapy are the treatment strategies adopted in patients with locally advanced or metastatic disease, not suitable for surgery (6). Etoposide, doxorubicin and cisplatin administered in association of mitotane (EDP-M) is the standard chemotherapeutic regimen (7,8). The efficacy of this regimen is overall limited, although a subgroup of patients can obtain a long-term survival (9). Non progressing patients after 6 cycles of EDP usually continue mitotane therapy till progression.

Mitotane exerts an adrenolytic activity requiring glucocorticoid supplementation, which is tapered on the basis of clinical assessment (10). Cortisone acetate or hydrocortisone are the recommended drugs for steroid replacement (1,5). Due to increased steroid clearance and increased cortisol-binding globulin levels induced by mitotane, a higher replacement dose is usually required than that prescribed for the management of Addison disease (1). Glucocorticoid replacement in patients taking mitotane is empirically done and may lead to either mild hypo- or hypercortisolism (11). Glucocorticoid administration, even at low doses, is associated with rapid bone loss and fragility (12). Glucocorticoids, in fact, negatively affect bone health via a complex mechanism that includes both a decrease in bone formation and an increase in bone resorption. Glucocorticoid-induced inhibition of bone formation involves multiple pathways, including a reduction in osteoblast proliferation via suppression of the growth factors BMP2 and TGFb1, upregulation of Wnt antagonists (Dkk-1, Wif-1, and Sost), and downregulation of the Wnt receptor complex (frizzled 4, 7, Dsh1, and Axin1). This results in strong suppression of osteoblast differentiation, maturation, and activity (13). Skeletal fragility is a frequent complication of Cushing syndrome, and fragility fractures may be the first clinical manifestation of the disease. However, improvement of bone mineral density was reported after resolution of hypercortisolism (14).

On these premises, a detrimental effect of steroid replacement on bone deserves attention in ACC patients on mitotane therapy. This issue is relevant since many patients are receiving mitotane after surgery in adjuvant setting and a number of patients with advanced disease are on mitotane therapy for a long time period. To the best of our knowledge, the effect of mitotane (and associated steroid replacement) on fracture risk has never been explored. Vertebral fractures (VFs) are the classical hallmark of glucocorticoid-induced osteoporosis and bone fragility (15,16), and are associated

with impaired quality of life and an increased risk of future fractures (17). The majority of VFs are mild and asymptomatic, and are diagnosed through a radiological exam. Vertebral morphometry is a quantitative method to identify VFs. It is based on the measurement of dorsal and lumbar vertebrae on lateral radiographic images (Morphometric X-ray Radiography, MRX) or on DEXA scans (Morphometric X-Ray Absorptiometry, MXA) (18). With the purpose to evaluate the frequency of VFs in ACC patients during mitotane therapy, we conducted a retrospective case-control study in 2 reference institutions for the management of ACC patients in Italy and France, respectively. The primary aim was to assess the frequency of VFs before and after mitotane treatment, secondary aims were: assessment of the proportion of patients with progression of VF or worsening of the spinal deformity index (SDI) during mitotane therapy, identification of risk factors for progression of VF and the impact of VFs on patient prognosis.

Patients and methods

Study design and objectives

This was a retrospective, multicentric, case-control study. Data of patients from the Azienda Socio Sanitaria Territoriale (ASST) Spedali Civili in Brescia (Italy) and Institute Gustave Roussy in Villejuif (France), meeting the eligibility criteria and recruited from January 2010 to January 2020, have been collected. Italian patients were the primary cohort and French patients the validation cohort. To be included in the study, the patients should have met the following eligibility criteria: age > 18 years; histological diagnosis of adrenocortical carcinoma; mitotane treatment alone or in association with chemotherapy for at least 6 months; availability of CT scans with sagittal view performed at baseline and during treatment. Exclusion criteria were the following: other malignancies except basal cell carcinoma or *in situ* cervical cancer diagnosed within 5 years before the diagnosis of ACC, concomitant diseases known to affect bone, previous or concomitant bisphosphonate therapy or other bone-active drugs except for vitamin D supplements, bone metastases. All patients received steroid supplementation, which consisted in cortisone acetate in Italian patients and hydrocortisone in the French patients. The equivalence between the 2 steroids is: hydrocortisone 20 mg = cortisone acetate 25 mg. The primary objective was to evaluate the prevalence of morphometric VFs during at least 6 months of treatment with mitotane ± chemotherapy in the overall series, and in the primary and validation cohorts. Secondary endpoints were the following: evaluation of proportion of patients with VF progression and worsening of the spinal deformity index during mitotane therapy, identification of bone fragility factors at baseline (i.e. hormone hypersecretion, age, sex, BMI, menopausal status, defined as age ≥60 years or no menses for 1 year in the absence of prior chemotherapy or tamoxifen use, or ovariectomy), and dosage of cortisol replacement therapy as independent risk factors of VF progression; prognostic role of VFs on overall survival in the subgroup of patients with metastatic disease.

In addition to ACC patients on mitotane, two different control groups were also included: 1) ACC patients not treated

with mitotane and 2) breast cancer (BC) patients treated with aromatase inhibitors (AI) in adjuvant setting for at least 2 years. BC patients had normal renal function, none of them were receiving previous or current treatment with anti-osteoporotic drugs (except for calcium and vitamin D) or glucocorticoids. Previous chemotherapy was permitted, but tamoxifen use was not. In the group 1) volumetric spine CT scans obtained at ACC diagnosis and during the follow up period were evaluated for VFs (at least every 6 months); in the group 2) CT scans were prospectively evaluated at baseline conditions, before the beginning of AI treatment, and at subsequent follow-up assessments.

All the ACC patients enrolled in the present study are registered in the ENS@T ACC registry approved by the local ethics committees of both Brescia and Villejuif. BC patients were included in a clinical study conducted at Medical Oncology and Breast Unit of ASST-Spedali Civili in Brescia (19, 20). All patients included had given their written informed consent.

Data collection and assessment of vertebral fractures

Demography data, comorbidities, clinical and pathological tumor characteristics and treatment information have been collected. Volumetric spine CT scans obtained before the beginning of mitotane treatment have been evaluated and compared to those obtained after at least 6 months of treatment. The sagittal reformatting view with bone window has been used for the analysis performed by the same experienced radiologists, following a standardized semi-quantitative morphometric approach applied to dorsal and lumbar vertebrae.

Vertebrae T4 to L4 were assessed for the presence of VFs according to the validated Genant's semi-quantitative method (21). The Genant Grades were defined as following: percentage reduction of the anterior, middle, and/or posterior height: grade 1 (mild fracture) 20-25%; grade 2 (moderate fracture) 25-40%; grade 3 (severe fracture) >40%. Wedge, biconcave and concave fractures were also separately identified. Prevalent VFs were analyzed at baseline. VFs of any grade present at follow up, but not at baseline, were considered new VFs. Progression of VFs was defined as the development of new/incident VFs (in patients with no VFs or in patients with previous VFs in other vertebrae) and/or the documented minimum 1-point increase in the Genant scoring in preexisting VFs during the period of follow-up.

A spinal deformity index (SDI), which has been demonstrated to be a good predictor of incident vertebral fractures, was calculated by summing in each patient the grade of each vertebra from T4 to L4 (22).

Radiographs were individually assessed simultaneously by a team of three experienced observers (MF, RG and CV), who were blinded for any patient characteristics. In case of a discrepancy in assessment, a consensus opinion was obtained.

Statistical analysis

Descriptive statistics were used to analyze patient clinical characteristics. Differences between categorical variables were assessed by chi-square or Fisher test when indicated. Whereas, continuous variables were compared through parametric (T-test) and non-parametric (Wilcoxon-test, Mann-Withney U-test) when indicated. Logistic regression model was employed to assess the odd ratios (ORs) and 95% confidence intervals (95% CIs) both in the univariate and multivariate analysis, with the lowest risk group as the reference group. Only factors that obtained $p<0.10$ in univariate analysis entered the multivariate model. OS curves have been calculated with Kaplan–Meier method and compared with log-rank test. Statistical significance has been set at $p < 0.05$. SPSS v23.0 software has been used for the statistical analyses (SPSS Inc., Chicago, IL).

Results

Patient characteristics

A total of 91 ACC consecutive patients, meeting the eligibility criteria and treated with mitotane entered the study: 46 were enrolled at the Medical Oncology Unit at ASST-Spedali Civili in Brescia (Italy) and 45 at the Endocrine Oncology and Nuclear Medicine Department, Gustave Roussy Cancer Institute in Villejuif (France). Patient and tumor characteristics are summarized in Table 1. Median age at the beginning of mitotane therapy was 51 years (range 18-72) in the Italian group, and 44 (range 17-78) in the French group. Median BMI was 24.3 (range 16.4-31.2) and 23.8 (range 18.9-36.9) in the two groups, respectively. Although not statistically significant, a higher percentage of Italian women had a postmenopausal status at diagnosis: 43.5%, versus 26.7% of the French counterpart ($p = 0.24$). Tumor hypersecretion at diagnosis was present in 29 Italian patients (63.0%) and 36 French patients (80.0%) ($p = 0.10$). In particular, cortisol hypersecretion was higher in the French cohort (68.9%) than in the Italian cohort (47.8%, p 0.05). Nine (18%) patients in the Italian and 19 (38%) in the French cohort had cortisol hypersecretion at the time of starting systemic antineoplastic therapy ($p = 0.04$).

At the time of the first evaluation, the proportions of patients with limited (stage I-II), locally advanced (stage III) or metastatic disease (stage IV) were 19.6%, 15.2% and 65.2% in the Italian group and 24.4%, 40.0% and 35.6% in the French series ($p = 0.01$). During the study period, 7 Italian and 19 French patients progressed from non-metastatic to metastatic disease.

In the whole population, 31 patients (34.1%) were treated with single agent mitotane (27 as adjuvant therapy 4 as palliative therapy), 43 patients (47.2%) with mitotane in association with EDP or EP (EDP/EP-M). Seventeen patients (18.7%), initially treated with mitotane in adjuvant setting, received also the EDP or the EP regimen at disease progression while continuing mitotane (these patients were counted in both groups). Comparing the two cohorts, a

larger number of patients was treated with mitotane monotherapy in the French cohort, whereas in the Italian one a higher number received concomitant mitotane and chemotherapy ($p = 0.008$ and $p = 0.0001$, respectively).

Hydrocortisone dose was converted to the equivalent cortisone acetate dose and the median supplementation dose was cortisone acetate 62.5 mg/day (range 12.5-150).

Patients were followed for a median time of 30.6 months. At the last follow-up examination, 40 patients (43.9%) were died. The median OS of the overall series was 24.2 months (range: 6.2-194.7).

Table 1 also describes the characteristics of patients not receiving mitotane therapy (control group 1). In particular, median age at diagnosis was 44 years (range 21 - 74), 71.4% of patients were females and tumor hypersecretion was present in 61.4% of cases.

The control group of BC patients consisted in 176 women (control group 2). Their median age was 64 years (range 30-74), all of them was in post-menopausal status (physiological or drug-induced) and the median BMI was 25.0 (range 16.5-42.2). Regarding histological subtype, 150 patients (85.2%) had a HR positive/HER2 negative whereas 26 patients (14.8%) had a HER2 positive/HR positive tumor. All of the considered patients (100%) underwent surgery for BC. Neoadjuvant or adjuvant chemotherapy were used in 6.3% and 36.4% of patient respectively. All of the patients received adjuvant hormone therapy, in particular 155 patients (88.1%) received AI alone, whereas 21 patients (11.9%) were treated with AI plus Luteinizing Hormone Releasing Hormone (LHRH). CT scans performed in each patient at baseline and every 6 months were prospectively evaluated.

Vertebral fractures in ACC patients at baseline

At baseline conditions, 13 out of 46 patients (28.3%) in the Italian group, 8 out of 45 patients (17.8%) in the French group, who subsequently received mitotane therapy and 3 out of 21 (14.3%) ACC patients not treated with mitotane had at least one prevalent VF. Among ACC patients treated with mitotane, 12 (26.1%) and 4 (8.9%) patients, respectively, had ≥1 mild VF, 4 (8.7%) and 2 (4.4%) had ≥1 moderate VF and 1 (2.2%) and 3 (6.7%) had ≥1 severe VF. Five Italian and 1 French patients had multiple VFs with different grades. Considering all patients together, VFs were found in 24 patients (21.4%) at baseline, 13 (11.6%) classified as mild, 7 (6.2%) moderate and 4 (3.6%) severe. Seven (6.2%) patients had multiple VFs with different grades. As depicted in the Table 2, baseline VFs significantly correlated with older age and sex, while no significant relationship was found with BMI, cortisol excess and, among women, menopausal status.

Vertebral fractures in the follow-up

In the patient subset treated with mitotane ± chemotherapy assessed after a median treatment time of 13.2 months (range 6.3- 48.3) in the Italian patients and 24.4 months (range 7.0-41.0) in the French ones, a significant increase in the frequency of VFs was seen in both series ($p = 0.043$ and $p = 0.047$, respectively) (Table 3). In particular, progression of VFs was observed in 18 Italian patients (39.1%), and in 13 French patients (28.9%).

Considering together the 2 series, 53 patients (58.2%) remained free from VF during the study period; baseline fractures remained stable in 7 (7.7%) patients, whereas a VF progression was seen in 31 (34.1%) patients (Table 3). Moreover, the total number of VFs of 37 at baseline (22 G1, 9 G2, 6 G3) increased to 113 after treatment (38 G1, 42 G2, 33 G3). The distribution of VF severity and SDI before and after mitotane ± chemotherapy is given in Figure 1. In particular, the proportions of moderate and severe VFs were 5.5% and 4.4% at baseline and 11% and 15.4% after mitotane. A worsening in SDI was seen in 18 (39.1%) Italian patients and in 13 (28.9%) French ones. In the whole series 34.1% of patients had a worsened SDI.

Conversely, in ACC patients not treated with mitotane there was only one new fracture in the follow-up and the increase in the proportion of patients with vertebral fractures was not statistically significant ($p = 0.50$) (Table 3). In the BC patient subset, the treatment with AIs led to a statistically significant rise in the proportion of patients with at least one VF (13.5% vs 20.2%, $p = 0.04$). Moreover, 13 patients developed new VFs (7.4%) and 5 patients had worsened VFs (2.8%). Figure 1 gives the distribution of VF severity and SDI before and after AIs in BC patients. The proportions of moderate and severe VFs were 5.6% and 0.6% at baseline and 8.4% and 1.7% after AI therapy and 11.9% of patients had a worsened SDI (Table 3).

Risk factors of VF progression in ACC patients receiving mitotane therapy

At univariate analysis, the following baseline characteristics: age ⩾48years, BMI ⩾24 and the presence of a VF were associated to an increased risk of vertebral fractures progression. Both the presence of baseline VFs and BMI maintained an independent association at multivariate analysis (odd ratio [OR] 4.2, 95%CI: 1.3-12.9, $p = 0.01$ and OR 2.6, 95% CI: 1.0-7.1, $p = 0.05$, respectively) (Table 4).

Relationship between VF progression and patient outcome

Among the 72 metastatic patients, 28 (38.9%) had VF progression after treatment. VF progression was associated with a poorer survival (median 20.9 vs 41.5 months, Hazard Ratio 0.50, 95%CI 0.26-0.95, $p = 0.032$) (Figure 2).

Discussion

This multicentric, retrospective study was designed to obtain data on the impact on bone fragility of systemic treatment

administered in patients with ACC. Patients with ACC undergoing mitotane +/- chemotherapy from 2 reference centers for this rare disease were included in the study. We also evaluated a control group of patients with ACC not receiving mitotane therapy and a series of breast cancer patients undergoing aromatase inhibitors, a treatment known to be associated with an increased frequency of fragility fractures.

This study demonstrates for the first time a progression of VFs in 34% of patients with ACC treated with mitotane during a median follow-up period of 30 months. A recent multicenter, multinational, retrospective study, showed that bone metastases in ACC patients are associated with high risk of adverse skeletal related events (23). In this patient series without bone metastases, we showed that also oncological treatment may contribute to an increased bone fragility and elevated fracture risk. The Italian and French patients were similar in terms of sex distribution and BMI at baseline conditions, however Italian patients were older and had a greater proportion of menopausal women than the French counterpart. With regard to tumor characteristics, ACCs in the Italian population were more advanced and less frequently hormone secreting than in the French one. Consequently, a greater proportion of Italian patients received chemotherapy in association with mitotane. These differences notwithstanding, a significant progression of VFs was demonstrated in both series considered separately.

About 23% of patients of patients treated with mitotane had at least one VF at baseline. Although this proportion is comparable to the VF frequency in the general population of healthy individuals (men and women) with $\geq$50 yr age (24), it was superior to that in the control patients with ACC who did not receive mitotane. Most of the patients treated with mitotane had advanced disease and this condition, even in the absence of bone metastases, predisposes for a higher bone turnover (25). The frequency of VFs consistently increased in patients treated with mitotane $\pm$ chemotherapy (42%) but not in untreated patients. Mitotane therapy was also associated to a consistent increase in the VF severity: grade 2-3 VFs according to the Genant scale increased from 40.5% at baseline to 66.4% after treatment and this observation is noteworthy, since moderate-severe VFs are clinically relevant (26). It is known that AI administration in women with breast cancer is associated with an increased risk of bone fragility and fractures and a bone loss preventive therapy with bisphosphonates or denosumab is currently recommended by international guidelines in this setting (16, 27, 28). Noteworthy, the absolute increase in VF after AI in breast cancer patients evaluated in this study (+6.7%) was lower than that observed in ACC patients submitted to mitotane (+18.7%) and VF progression occurred in 2.8% versus 6.6% of patients, respectively. This observation is relevant and provides a measure of the considerable deterioration of bone health observed in patients on mitotane therapy.

We also performed some exploratory analyses to evaluate the impact of other well-known parameters associated with bone fragility on the fracture risk of the patients included in the study. At baseline, only age and sex were associated with the presence of VFs. Other bone fragility parameters such as BMI, menopause and cortisol hypersecretion at diagnosis failed to show a significant relationship. Older age and the presence of at least one VF at baseline

significantly correlated with an increased risk of VF progression after mitotane treatment in univariate analysis. The presence of fractures at baseline maintained the statistical significance in multivariate analysis while age just failed to enter the model. These data are consistent with what is observed in postmenopausal osteoporosis where both age and history of previous fractures strongly predict fracture risk regardless of bone mineral density (29). However, in this series high BMI appeared to be associated with an increased fracture risk after mitotane therapy and this is the opposite of what is observed in postmenopausal osteoporosis where low BMI is an independent risk factor (30,31). The association between adiposity and bone fragility is complex (32). On one hand, obesity has a protective role in relation to the higher concentrations of estrogens due to higher aromatase activity, on the other hand it is associated with detrimental effects on bone quality via several mechanisms, including alteration of bone regulating hormones, increased oxidative stress and inflammation, and altered bone cell metabolism (32). In a recent cross-sectional study, elevated fat body mass was found to have a direct relationship with fracture risk in women under AI therapy (19). A plausible mechanism is that AIs induce a profound inhibition of estrogen synthesis leading to a loss of estrogen protection against fragility-related fracture and allowing the deleterious effect of adiposity to prevail. Mitotane has been shown to inhibit the aromatase enzyme (33) and, similarly to what we observed in women with breast cancer under AIs, this mechanism may explain the correlation between high BMI and VF. Another possible alternative is that the deleterious effect of adipose tissue on bone quality in patients with high BMI may be synergistic with steroid supplementation associated to mitotane therapy. However, the dose of steroids did not show any correlation with VF and this suggests that steroid supplementation does not play a major role on the bone fragility of patients on mitotane therapy.

Finally, the present study also showed that, in patients with advanced ACC without overt bone metastases, the occurrence of VFs was associated with poorer prognosis. This observation is in line with what observed both in the general population (34) and in cancer patients undergoing specific antineoplastic therapy (35).

The relatively high number of patients enrolled and the significant increase in the number and severity of VFs, which occurred after treatment both in the exploratory and validation series, are strengths of this study. The retrospective nature and the different observation period to which patients were subjected are the main limitations.

In conclusions, this study demonstrated for the first time that ACC patients, which are submitted to mitotane therapy, are at high risk of developing fragility fractures. Previous fractures and high BMI are independent risk factors. These data should be taken into consideration in order to adopt appropriate preventive measures, such as the prescription of bone resorption inhibitors.

Data availability

Some data generated and analyzed during this study are included in this published article.

References

1. Fassnacht M, Assie G, Baudin E, et al. Adrenocortical carcinomas and malignant phaeochromocytomas: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020;31(11):1476-90.
2. Baudin E, Roussy ETBoG. Adrenocortical carcinoma. Endocrinol Metab Clin North Am. 2015;44(2):411-34.
3. Terzolo M, Angeli A, Fassnacht M, et al. Adjuvant mitotane treatment for adrenocortical carcinoma. N Engl J Med. 2007;356(23):2372-80.
4. Berruti A, Grisanti S, Pulzer A, et al. Long-Term Outcomes of Adjuvant Mitotane Therapy in Patients With Radically Resected Adrenocortical Carcinoma. J Clin Endocrinol Metab. 2017;102(4):1358-65.
5. Fassnacht M, Dekkers OM, Else T, et al. European Society of Endocrinology Clinical Practice Guidelines on the management of adrenocortical carcinoma in adults, in collaboration with the European Network for the Study of Adrenal Tumors. Eur J Endocrinol. 2018;179(4):G1-G46.
6. Terzolo M, Daffara F, Ardito A, et al. Management of adrenal cancer: a 2013 update. J Endocrinol Invest. 2014;37(3):207-17.
7. Berruti A, Terzolo M, Sperone P, et al. Etoposide, doxorubicin and cisplatin plus mitotane in the treatment of advanced adrenocortical carcinoma: a large prospective phase II trial. Endocr Relat Cancer. 2005;12(3):657-66.
8. Fassnacht M, Terzolo M, Allolio B, et al. Combination chemotherapy in advanced adrenocortical carcinoma. N Engl J Med. 2012;366(23):2189-97.
9. Laganà M, Grisanti S, Cosentini D, et al. Efficacy of the EDP-M Scheme Plus Adjunctive Surgery in the Management of Patients with Advanced Adrenocortical Carcinoma: The Brescia Experience. Cancers (Basel). 2020;12(4).
10. Chortis V, Taylor AE, Schneider P, et al. Mitotane therapy in adrenocortical cancer induces CYP3A4 and inhibits 5α-reductase, explaining the need for personalized glucocorticoid and androgen replacement. J Clin Endocrinol Metab. 2013;98(1):161-71.
11. Mazziotti G, Formenti AM, Frara S, et al. MANAGEMENT OF ENDOCRINE DISEASE: Risk of overtreatment in patients with adrenal insufficiency: current and emerging aspects. Eur J Endocrinol. 2017;177(5):R231-R48.
12. Canalis E. Mechanisms of glucocorticoid-induced osteoporosis. Curr Opin Rheumatol. 2003;15(4):454-7.
13. Dalla Volta A, Mazziotti G, Maffezzoni F, et al. Bone Mineral Density and FRAX Score May Not Predict Fracture Risk in Patients With Cancer Undergoing Hormone Deprivation Therapies. J Clin Oncol. 2020;38(29):3363-3366.
14. Mazziotti G, Delgado A, Maffezzoni F, Formenti A, Giustina A. Skeletal Fragility in Endogenous Hypercortisolism. Front Horm Res. 2016;46:66-73.
15. Cooper C, Atkinson EJ, O'Fallon WM, Melton LJ. Incidence of clinically diagnosed vertebral fractures: a population-based study in Rochester, Minnesota, 1985-1989. J Bone Miner Res. 1992;7(2):221-7.
16. Pedersini R, Monteverdi S, Mazziotti G, et al. Morphometric vertebral fractures in breast cancer patients treated with adjuvant aromatase inhibitor therapy: A cross-sectional study. Bone. 2017;97:147-52.
17. Adachi JD, Adami S, Gehlbach S, et al. Impact of prevalent fractures on quality of life: baseline results from the global longitudinal study of osteoporosis in women. Mayo Clin Proc. 2010;85(9):806-13.
18. Oei L, Rivadeneira F, Ly F, et al. Review of radiological scoring methods of osteoporotic vertebral fractures for clinical and research settings. Eur Radiol. 2013;23(2):476-86.
19. Pedersini R, Amoroso V, Maffezzoni F, et al. Association of Fat Body Mass With Vertebral Fractures in Postmenopausal Women With Early Breast Cancer Undergoing Adjuvant Aromatase Inhibitor Therapy. JAMA Netw Open. 2019;2(9):e1911080. Published 2019 Sep 4.
20. Monteverdi S, Pedersini R, Gallo F, et al. The Interaction of Lean Body Mass With Fat Body Mass Is Associated With Vertebral Fracture Prevalence in Women With Early Breast Cancer Undergoing Aromatase Inhibitor Therapy. JBMR Plus. 2020;5(2):e10440. Published 2020 Dec 21.
21. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res. 1993;8(9):1137-48.
22. Kerkeni S, Kolta S, Fechtenbaum J, Roux C. Spinal deformity index (SDI) is a good predictor of incident vertebral fractures. Osteoporos Int. 2009;20(9):1547-52.
23. Berruti A, Libè R, Laganà M, et al. Morbidity and mortality of bone metastases in advanced adrenocortical carcinoma: a multicenter retrospective study. Eur J Endocrinol. 2019;180(5):311-20.
24. Kendler DL, Bauer DC, Davison KS, et al. Vertebral Fractures: Clinical Importance and Management. Am J Med. 2016;129(2):221.e1-10.
25. Berruti A, Torta M, Piovesan A, et al. Biochemical picture of bone metabolism in breast cancer patients with bone metastases. Anticancer Res. 1995;15(6B):2871-2875.
26. Fechtenbaum J, Cropet C, Kolta S, Horlait S, Orcel P, Roux C. The severity of vertebral fractures and health-related quality of life in osteoporotic postmenopausal women. Osteoporos Int. 2005;16(12):2175-9.

27. Coleman R, Hadji P, Body JJ, et al. ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Bone health in cancer: ESMO Clinical Practice Guidelines. Ann Oncol. 2020 Dec;31(12):1650-1663.
28. Genant HK, Libanati C, Engelke K, et al. Improvements in hip trabecular, subcortical, and cortical density and mass in postmenopausal women with osteoporosis treated with denosumab. Bone. 2013;56(2):482-488.
29. Kanis JA, Cooper C, Rizzoli R, Reginster JY, on behalf of the Scientific Advisory Board of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) and the Committees of Scientific Advisors and National Societies of the International Osteoporosis Foundation (IOF). Executive summary of the European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Calcif Tissue Int. 2019;104(3):235-8.
30. Villareal DT, Apovian CM, Kushner RF, Klein S, Nutrition ASf, NAASO TeOS. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, The Obesity Society. Am J Clin Nutr. 2005;82(5):923-34.
31. Reid IR, Ames R, Evans MC, et al. Determinants of total body and regional bone mineral density in normal postmenopausal women--a key role for fat mass. J Clin Endocrinol Metab. 1992;75(1):45-51.
32. Shapses SA, Pop LC, Wang Y. Obesity is a concern for bone health with aging. Nutr Res. 2017;39:1-13.
33. Wójtowicz AK, Kajta M, Gregoraszczuk E. DDT- and DDE-induced disruption of ovarian steroidogenesis in prepubertal porcine ovarian follicles: a possible interaction with the main steroidogenic enzymes and estrogen receptor beta. J Physiol Pharmacol. 2007;58(4):873-85.
34. Tran T, Bliuc D, Pham HM, et al. A Risk Assessment Tool for Predicting Fragility Fractures and Mortality in the Elderly. J Bone Miner Res. 2020;35(10):1923-34.
35. Shao YH, Moore DF, Shih W, Lin Y, Jang TL, Lu-Yao GL. Fracture after androgen deprivation therapy among men with a high baseline risk of skeletal complications. BJU Int. 2013;111(5):745-52.

Tables

Table 1. Patient characteristics.

	Patients treated with Mitotane				Patients NOT treated with mitotane
	Italian	**French**	***p*-value**	**Total**	
N°	46	45		91	21
Age Median (range)	51 (18-72)	44 (17-78)	0.14	48 (17-78)	44 (21 - 74)
Sex, N (%) Male Female	18 (39.1) 28 (60.9)	18 (40.0) 27 (60.0)	1.00	36 (39.6) 55 (60.4)	6 (28.6) 15 (71.4)
BMI Median (range) Not available, N (%)	24.3 (16.4-31.2) 0 (0.0)	23.8 (18.9-36.9) 3 (6.6)	0.59	24.0 (16.0-37.0) 3 (3.3)	 21 (100%)
Menopausal status N (%) Not available, N (%)	20 (43.5) 0 (0.0)	12 (26.7) 3 (6.7)	0.24	32 (35.1) 3 (3.3)	32 (35.1) 3 (3.3)
Tumor secretory status at diagnosis, N (%) Secreting tumors, Cortisol excess (± other hormones) Androgens only Other Non secreting tumors	29 (63.0) 22 (47.8) 5 (10.9) 1 (2.2) 17 (37.0)	36 (80.0) 31 (68.9) 5 (11.1) 0 (0.0) 9 (20)	0.10 **0.05** 1.00	65 (71.4) 53 (58.2) 10 (11.1) 1 (1.1) 26 (28.6)	11 (52.4) 11 (52.4) 0 (0.0) 0 (0.0) 10 (47.6)
Cortisol hypersecretion at the time of mitotane starting, N (%)	9 (18.0)	19 (38.0)	**0.04**	28 (56.0)	
ENSAT stage, N (%) I-II III IV	9 (19.6) 7 (15.2) 30 (65.2)	11 (24.4) 18 (40.0) 16 (35.6)	0.62 **0.01** **0.006**	20 (22.0) 25 (27.5) 46 (50.5)	14 (66.7) 7 (33.3) 0 (0.0)
Treatment administered, N (%) Only mitotane Adjuvant mitotane and then chemotherapy plus mitotane Concomitant chemotherapy plus mitotane	10 (21.7) 7 (15.2) 29 (63.0)	21 (46.7) 10 (22.2) 14 (31.1)	**0.008** 0.10 **0.0001**	31 (34.1) 17 (18.7) 43 (47.2)	
Mitotane therapeutic range attainment, N (%) Not available	28 (60.9) 0 (0.0)	29 (64.4) 3 (6.7)	0.50	57 (62.6) 3 (3.3)	
Cortisone acetate supplementation *, N (%) Median mg/day	46 (100) 50 (range 12.5-150)	45 (100) 62.5 (range 25-100)	1.00 **0.037**	91 (100) 62.5 (range 12.5-150)	

BMI, Body Mass Index; NA not available.

*Italian patients were supplemented with cortisone acetate whereas French patients with hydrocortisone. Hydrocortisone dose was converted in the equivalent cortisone acetate dose.

Table 2. Correlations between risk factors of bone fragility and vertebral fractures at baseline.

VFs	No	Yes	*p*-value
Age at mitotane start, N/Tot (%)			**0.004**
<48 years	51/112 (45.5)	6/112 (5.4)	
≥48 years	37/112 (33.0)	18/112 (16-1)	
Sex, N/Tot (%)			**0.05**
Male	29/112 (25.9)	13 (11.6)	
Female	59/112 (52.7)	11 (9.8)	
BMI, N/Tot (%)			0.07
<24	37/88 (42.0)	7/88 (8.0)	
≥24	30/88 (34.1)	14/88 (15.9)	
Cortisol excess*, N/Tot (%)			0.11
Yes	44/112 (39.3)	16/112 (14.3)	
No	44/112 (39.3)	8/112 (7.1)	
Menopausal status, N/Tot (%)			0.62
Yes	33/66 (50.0)	6/66 (9.1)	
No	23/66 (34.8)	4/66 (6.1)	

VFs Vertebral Fractures.

*at the time of starting mitotane therapy

Table 3. Assessment of vertebral fractures on CT scan at baseline and after at least 6 months of mitotane± chemotherapy.

	ACC patients treated with Mitotane± chemotherapy						**ACC patients NOT treated with mitotane**		**Breast cancer patients treated with AI**	
	Italian patients		**French patients**		**IT+FR patients**					
	Baseline	**After M±CHT**	**Baseline**	**After M±CHT**	**Baseline**	**After M±CHT**	**Baseline**	**After**	**Baseline**	**After AI**
VFs, N/Tot (%)										
No VFs	33/46 (71.7)	24/46 (52.2)	37/45 (82.2)	29/45 (64.4)	70/91 (76.9)	53/91 (58.2)	18/21 (85.7)	17/21 (80.9)	154/176 (87.5)	141/176 (80.1)
VFs	13/46 (28.3)	22/46 (47.8)	8/45 (17.8%)	16/45 (35.6)	21/91 (23.1)	38/91 (41.8)	3/21 (14.3)	4/21 (19.1)	22/176 (12.5)	35/176 (19.9)
	p* 0.04		***p* 0.04***		***p* 0.005***		*p* 0.50*		***p* 0.04***	
New VFs, N/Tot (%)		17/46 (36.9)		13/45 (28.9)		30/91 (32.9)		1/21 (4.7)		13/176 (7.4)
Worsened VFs, N/Tot (%)		4/46 (8.7)		2/45 (4.4)		6/91 (6.6)		0/21 (0.0)		5/176 (2.8)
Both new and worsened VFs, N/Tot (%)		3/46 (6.5)		2/45 (4.4)		5/91 (5.5)		0/21 (0.0)		2/176 (1.1)
Stable VFs, N/Tot (%)		4/46 (8.7)		3/45 (6.7)		7/91 (7.7)		3/21 (14.3)		19/176 (10.8)
SDI, mean (±SD)	0.89 (±3.16)	2.20 (±4.63)	0.38 (±0.91)	2.67 (±5.29)	0.64 (±2.34)	2.43 (±4.94)	0.19 (±0.51)	0.29 (±0.64)	0.25 (±0.74)	0.39 (±0.95)
	***p* 0.03**		***p* 0.03**		***p* 0.002**		*p* 0.33		***p* 0.0001**	
SDI≥2, N/Tot (%)										
No	40/46 (87.0)	31/46 (67.4)	40/45 (88.9)	31/45 (68.9)	80/91 (87.9)	62/91 (68.1)	20/21 (95.2)	19/21 (90.5)	161/176 (91.5)	156/176 (88.6)
Yes	6/46 (13.0)	15/46 (32.6)	5/45 (11.1)	14/45 (31.1)	11/91 (12.1)	29/91 (31.9)	1/21 (4.7)	2/21 (9.5)	15/176 (8.5)	20/176 (11.4)
	***p* 0.02**		***p* 0.02**		***p* 0.001**		*p* 0.10		*p* 0.24	
Worsened SDI, N/Tot (%)		18/46 (39.1)		13/45 (28.9)		31/91 (34.1)		1/21 (4.7)		21/176 (11.9)

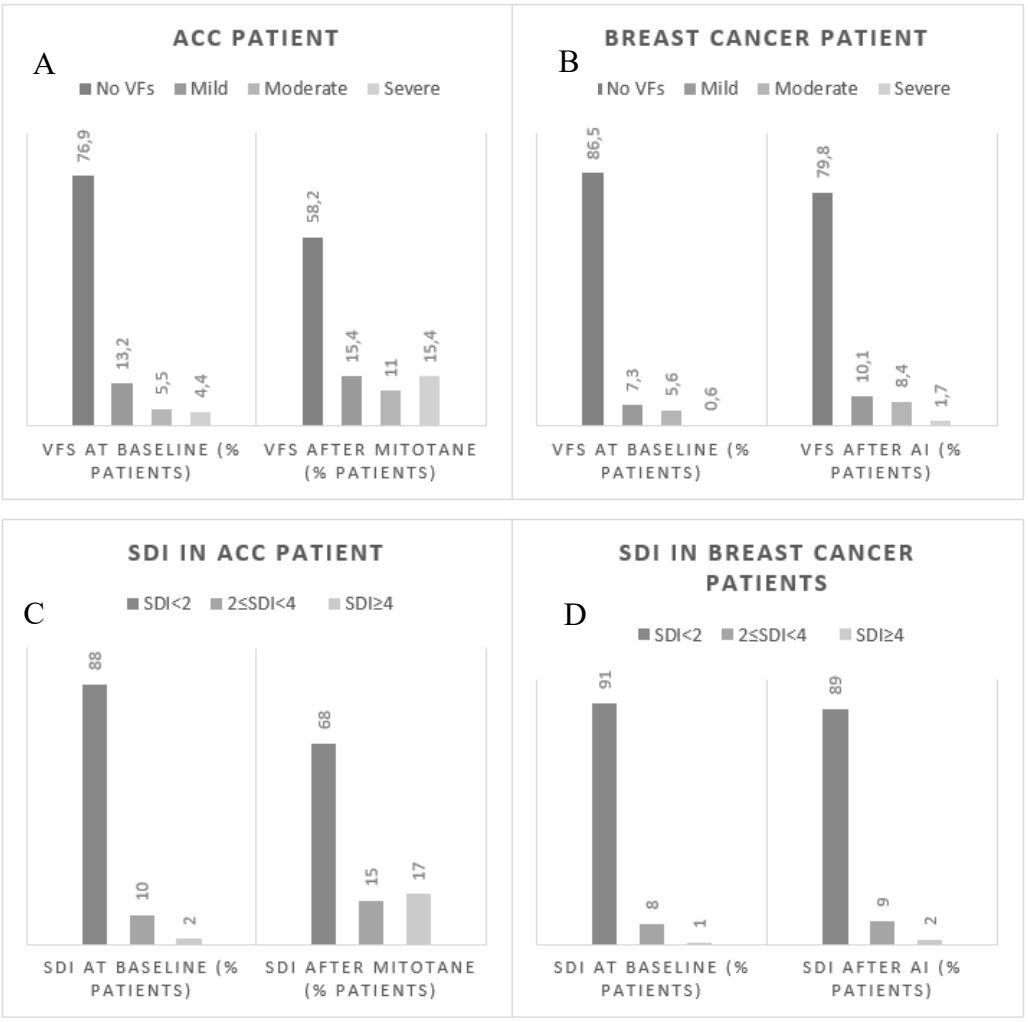
*One tailed chi square. Pt: patients, VFs: vertebral fractures. CHT: chemotherapy

Table 4. Correlations between VF progression and risk factors of bone fragility according to univariate and multivariate analyses.

	VF progression		Univariate analysis		Multivariate analysis	
	Yes	**No**	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**
Age, N/Tot (%)						
<48years	9/31 (29.0)	35/60 (58.3)	1	**0.010**	1	0.07
≥48years	22/31 (71.0)	25/60 (41.7)	3.4 (1.4-8.7)		2.5 (0.9-7.0)	
Sex, N/Tot (%)						
Male	12/31 (38.7)	24/60 (40.0)	1	0.90		
Female	19/31 (61.3)	36/60 (60.0)	1.1 (0.4-2.6)			
BMI, N/Tot (%)						
<24	10/31 (32.2)	23/57 (40.4)	1	**0.02**	1	**0.05**
≥24	21/31 (67.8)	34/57 (59.6)	3.1 (1.2-7.8)		2.6 (1.0-7.1)	
Menopausal status (total number of patients 51), N/Tot (%)				0.11		
Yes	14/18 (77.8)	18/33 (54.5)	2.9 (0.8-10.8)			
No	4/18 (22.2)	15/33 (45.5)	1			
Mitotane in range, N/Tot (%)				0.33		
Yes	18/31 (58.1)	39/57 (68.4)	1			
No	13/31 (41.9)	18/57 (31.6)	1.6 (0.6-3.9)			
Steroid replacement dose, N/Tot (%)				0.52		
≤62.5 mg/day	18/31 (58.1)	39/60 (65.0)	1			
>62.5 mg/day	13/31 (41.9)	21/60 (35.0)	1.3 (0.6-3.3)			
Chemotherapy, N/Tot (%)				0.23		
Yes	23/31 (74.2)	37/60 (61.7)	1.8 (0.7-4.7)			
No	8/31 (25.8)	23/60 (38.3)	1			
VFs at baseline, N/Tot (%)				**0.001**		**0.01**
Yes	14/31 (45.2)	7/60 (11.7)	6.2 (2.2-18.0)		4.2 (1.3-12.9)	
No	17/31 (54.8)	53/60 (88.3)	1		1	

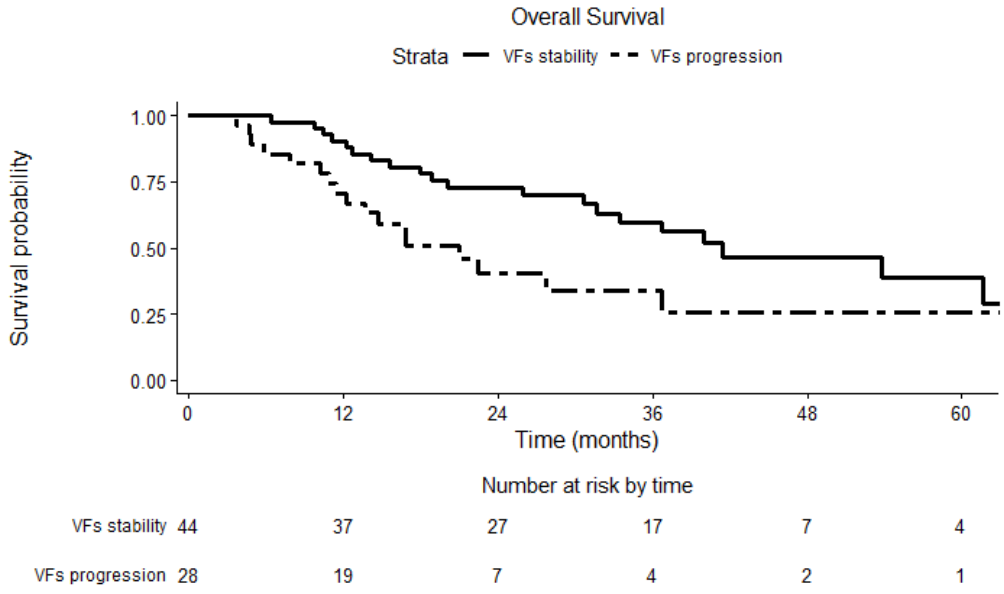
Figures

Figure 1. A: Proportion of ACC patients divided on the bases of the worst VF grade (mild, moderate, severe) at baseline and after at least 6 months of mitotane±chemotherapy. B: Proportion of BC patients divided on the bases of the worst VF grade (mild, moderate, severe) at baseline and after a median time of AI treatment of 18 months. C: Distribution of Skeletal Deformity Index (SDI)* at baseline and after treatment with mitotane±chemotherapy in ACC patients (proportion of patients). D: Distribution of SDI* at baseline and after treatment with AI in BC patients (proportion of patients).



CHT chemotherapy.
*SDI was calculated by summing in each patient the grade of each vertebra from T4 to L4. In theory, the SDI value can vary between 0 (no fracture) and 39 (all the assessed vertebrae are grade 3) (20).

Figure 2. Overall Survival in metastatic patients on the basis of VFs progression.



456