Am J Cancer Res 2024;14(4):1802-1814
www.ajcr.us /ISSN:2156-6976/ajcr0155726

Review Article

The effect of adjuvant mitotane therapy of the adrenocortical carcinoma on the endometrium and its clinical consequences in menstruating women. Literature review and authors' own experiences

Piotr Szkodziak[1], Filip Szkodziak[1], Agnieszka Korolczuk[2], Ewa Obel[3], Sławomir Woźniak[1], Tomasz Paszkowski[1]

[1]3rd Chair and Department of Gynaecology, Medical University of Lublin, Lublin, Poland; [2]Chair and Department of Clinical Pathomorphology, Medical University of Lublin, Lublin, Poland; [3]Chair and Department of Endocrinology, Medical University of Lublin, Lublin, Poland

Received January 31, 2024; Accepted March 31, 2024; Epub April 15, 2024; Published April 30, 2024

Abstract: Adrenocortical carcinoma (ACC) is a malignant tumour that originates from the adrenal cortex. It is a highly aggressive cancer characterised by a poor prognosis with an annual incidence estimated to be up to 2 cases per million. In the adult population, ACC is diagnosed typically between 40 and 50 years of age, more often in women. Complete surgical resection of the tumour is the primary treatment method for ACC. Unfortunately, despite properly performed adrenalectomy, regional recurrences or distant metastases are detected in up to 90% of the patients. For that reason, adjuvant therapy is recommended. Mitotane is the most effective adrenal-specific agent used in adjuvant and palliative therapy. Two menstruating patients, after adrenalectomy due to ACC, during adjuvant mitotane therapy, have been included in the study. The study aimed to assess the effect of mitotane therapy on the endometrium and its clinical consequences, based on the analysis of these two cases and a review of the literature. It seems that menorrhagia may be expected during adjuvant mitotane therapy of ACC in menstruating women. Heavy uterine bleeding during menstruation may appear several months after the beginning of therapy. The likely mechanism for heavy menstrual bleeding is complex. Menorrhagia can occur due to the toxic effect of mitotane in the form of a haemorrhagic diathesis, while long-term treatment (over ten months) can lead to relative hypoestrogenism resulting in endometrial hyperplasia. Clinical signs of hypoestrogenism during mitotane treatment, have been described (including pre-puberty girls) and should be considered as a side-effect of the therapy. Menorrhagia may lead to severe anaemia, so this should be considered when planning mitotane treatment. Continuous gestagen therapy is helpful in the treatment of the above disorders. After over 60 years of experience with mitotane usage, knowledge about it is still insufficient, and further studies are required.

Keywords: Mitotane, adrenocortical carcinoma, ACC, menorrhagia

Introduction

Adrenocortical carcinoma (ACC) is a malignant tumour that originates from the adrenal cortex [1]. It is a rare disorder with an annual incidence estimated to be up to 2 cases per million [2-4]. In the adult population, ACC occurs typically between 40 and 50 years of age, more often among women (55-65%) [4-7].

ACC is a highly aggressive cancer characterised by a poor prognosis. The median overall survival time varies from 38 to 56 months. The prognosis depends on the tumour stage and completeness of surgical treatment [8-10]. The five-year survival rate is 66-82% for stage I, 58-64% for stage II, 24-50% for stage III, and 0-17% for stage IV [11-14].

Even though ACC usually presents as a sporadic tumour, it could be observed with an increased frequency in hereditary cancer syndromes such as Li Fraumeni syndrome, familial adenomatous polyposis, multiple endocrine neoplasia type 1, Lynch syndrome and neurofibromatosis type 1 [4, 15].

https://doi.org/10.62347/QKWF9884

Generally, ACCs may be classified as functional (hormone-secreting) and non-functional tumours [5]. Functional ACCs are more frequent in females and overall account for 41-57% of all cases. These lesions are characterised mainly by hypercortisolaemia with symptoms of Cushing's syndrome, hyperandrogenaemia and hyperaldosteronism [16-19]. Other symptoms include abdominal pain, abdominal discomfort, hirsutism, weight change and asthenia [10, 16, 19]. On the other hand, non-functional ACCs are usually discovered incidentally by imaging procedures conducted for unrelated medical problems [20].

An adrenocortical adenoma is a common disorder, which diagnosis is often easy and straightforward, whereas the diagnosis of a rare ACC could cause difficulties [13, 21].

The diagnostic procedures of any adrenal tumours should include both laboratory workup and the use of cross-sectional imaging techniques. Laboratory workup includes mainly the assessment of serum levels of cortisol, aldosterone, corticotrophin, catecholamine, dehydroepiandrosterone-sulfate and sex steroid hormones [21]. Nowadays, the most frequently used imaging technique in the case of adrenal tumours is computed tomography (CT). The second-line tests consist of functional imaging by positron emission tomography with ^{18}F-fluorodeoxyglucose and magnetic resonance imaging [4, 23].

Biopsy of adrenal mass is rarely indicated. It has low diagnostic sensitivity and does not affect patient outcomes. Furthermore, it is associated with serious complications, including needle track metastases, exposing patients to unnecessary risks [21-25].

Complete surgical resection of the tumour is the only curative treatment method for ACC [26, 27]. The goal of surgical treatment is to achieve negative margins resection (R0), thus minimising the risk of recurrences [8, 9, 28, 29]. For this purpose, resection of the adrenal glands, lymph nodes and large veins should be performed en bloc [1, 22, 23, 30].

Open adrenalectomy is considered to be the gold standard in the surgical management of ACC [28, 31]. It enables optimal examination of the peritoneal cavity as well as provides easy access to the main vessels (renal artery, renal vein, aorta, inferior vena cava) and lymph nodes [22, 32]. Laparoscopic adrenalectomy (traditional, retroperitoneal, robot-assisted) could be performed in the case of tumours without signs of invasion, extensive lymphadenopathy, and distant metastases [33-36]. Despite the reduction of hospitalisation time, it may carry a risk associated with tumour spillage, as well as reduce the chance of obtaining negative margins resection and conducting comprehensive lymphadenectomy, especially for large tumours [28, 29, 36-38].

Unfortunately, despite properly performed adrenalectomy, regional recurrences or distant metastases are detected in up to 91% of the patients [39]. For that reason, adjuvant therapy is recommended. Mitotane is the most effective adrenal-specific agent that is used both in adjuvant and palliative therapy [40-43].

Mitotane's mode of action is not entirely explained. High mitotane doses cause adrenal cortex atrophy (destruction of the zona reticularis and fasciculata), inevitably resulting in a steroid secretion disorder. It seems that cytotoxicity is the result of oxidative stress induction [41, 44-48]. Moreover, mitotane inhibits cortisol synthesis by blocking the cholesterol sidechain and increasing the serum concentrations of the Corticosteroid Binding Globulin (CBG) and Sex Hormone Binding Globulin (SHBG), resulting in a reduction of the concentration of the hormones [49, 50].

Adjuvant mitotane therapy should begin within 12 weeks after surgery and be continued for 2-5 years if well tolerated [10, 51, 52]. The side effects mainly concern the digestive (vomiting, nausea, diarrhoea) and neurological systems (vertigo, lethargy, depression, ataxia). Moreover, symptoms of adrenal insufficiency and coagulopathy during adjuvant mitotane therapy were reported [1, 10].

Methods

This literature review is based on analysis of available literature indexed in MEDLINE, Cochrane and PubMed bases. It was conducted independently by two reviewers (PS and FS) between June 2023 and December 2023. The keywords used during analysis were the combinations of mitotane AND (adrenal cortical can-

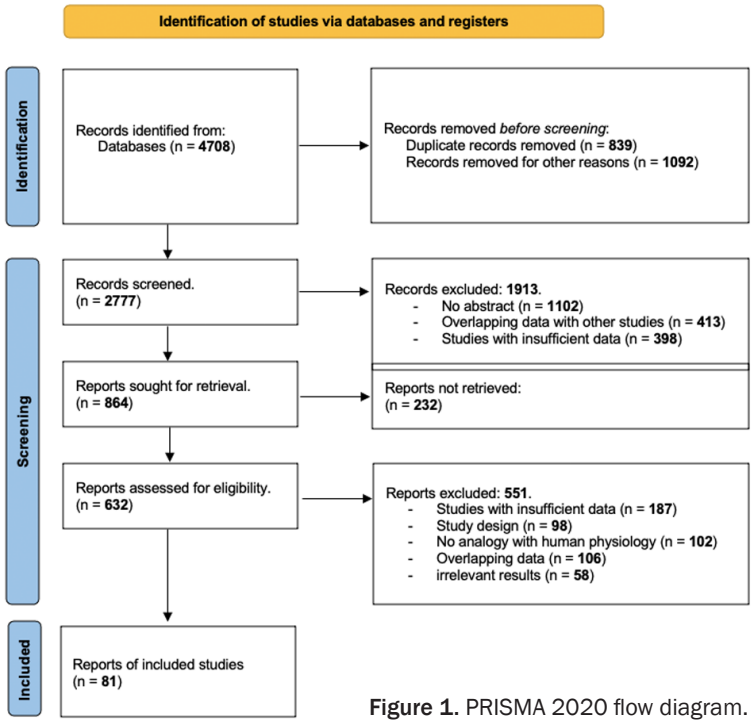

Figure 1. PRISMA 2020 flow diagram.

cer OR ACC OR menorrhagia OR metrorrhagia OR uterine bleeding OR hypoestrogenism OR relative hypoestrogenism OR endometrial hyperplasia OR side effect OR toxic effect OR expected after-effect OR expected consequence OR treatment OR adjuvant therapy OR staging OR ovarian function). The search was restricted to articles published in English from 1972 to the present. English written and relevant to the topic abstracts were chosen. Full articles were critically studied and analysed in detail.

Furthermore, to better understand and illustrate the effect of mitotane therapy on the endometrium, in addition to the literature review, an analysis of two cases was conducted. The study was approved by the Local Bioethics Committee (KE-0254/42/2020). The written agreement of both patients described in case reports was obtained.

Results

A total of 864 records from 1959-2023 were retrieved following the screening. 632 articles were assessed for eligibility. A final sample of 81 articles published between 1972 and 2023 was chosen for inclusion in the review (**Figure 1**).

Two menstruating patients, after adrenalectomy (in 2010 and 2019) because of ACC, during adjuvant mitotane therapy have been included in the study.

Case 1

A 33-year-old patient (Gravida 0, Parity 0) with a non-functional tumour in the right adrenal gland (78 × 68 × 82 mm) showing weak contrast enhancement in Computed Tomography Angiography (CTA) (**Figure 2A**, **2B**) underwent right-sided adrenalectomy. After surgical treatment of the tumour, the patient was qualified for adjuvant mitotane therapy with a therapeutic window of 14-20 mg/l (maximum mitotane serum level during therapy - 22.3 mg/l) (**Table 1**). After ten months of treatment, menorrhagia occurred. In the transvaginal ultrasound scan (TVUS) performed on the 10th day of the cycle widened to 15.2 mm, and heterogeneous endometrium (suggesting the presence of a polyp) was observed. The patient was qualified for hysteroscopy with endometrial biopsy. Microscopic examination of biopsied material revealed endometrium with an increase in the gland-to-stroma ratio with irregularities in gland shape and variation in gland size. Some glands were dilated and cystic (**Figure 3A**, **3B**). Glandular epithelium showed columnar cells with amphophilic cytoplasm, pseudostratified nuclei with evenly dispersed chromatin, indistinct nucleoli and focal mitotic activity. Signs of atypia were not seen. There was a moderate amount of intervening stroma between the glands consisting of small, oval cells with scanty cytoplasm. After hysteroscopy, menorrhagia continued, and the patient was re-hospitalized with a diagnosis of iron-deficiency anaemia. The patient was treated with red blood cell concentrate intravenous/oral iron supplementation. The lowest haemoglobin (Hb) level during bleeding episodes was 8.9 g/dL (**Table 2**). To prevent endometrial hyperplasia and the associated clinical consequences, the patient was qualified for the levonorgestrel-releasing intrauter-

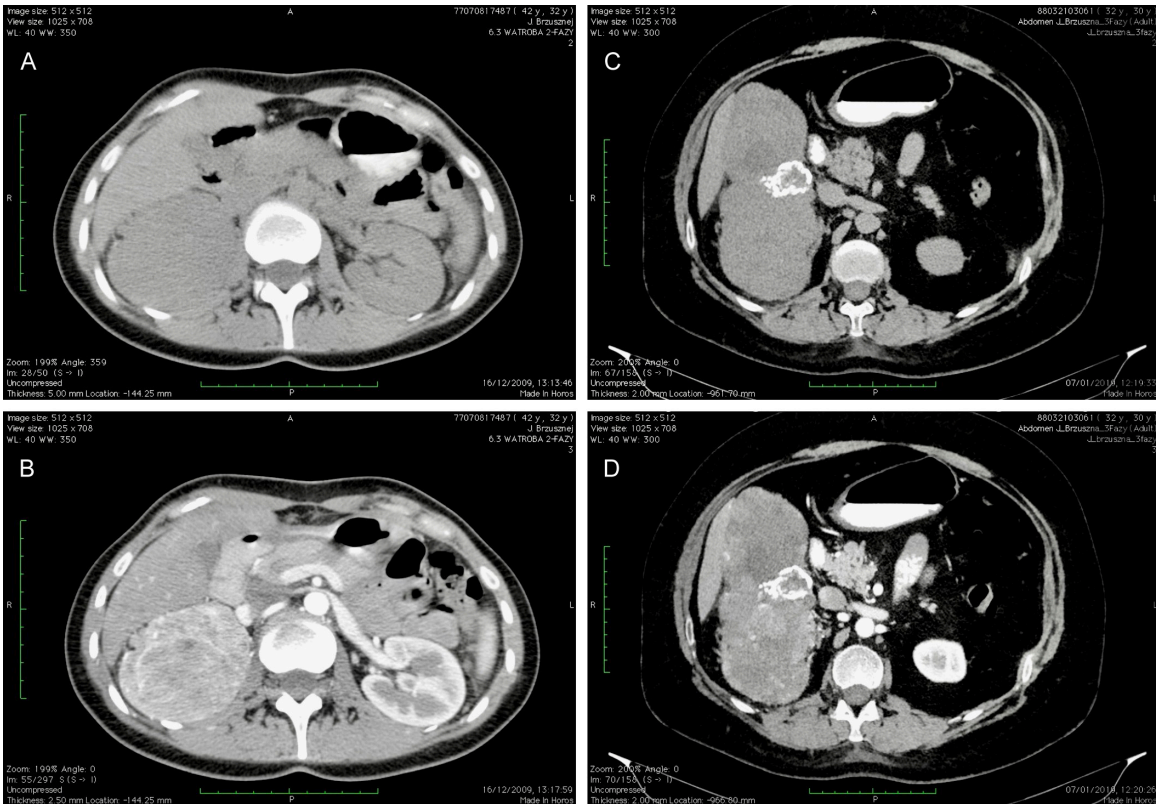

Figure 2. A. CT scan of the abdomen (transverse cross-section) in Case 1. In the area of the right adrenal gland, an oval tumour with a homogeneous structure is visible. B. CT scan of the abdomen (transverse cross-section) in Case 1. The tumour shows weak contrast enhancement. C. CT scan of the abdomen (transverse cross-section) in Case 2. In the areas of the right adrenal gland and right epigastrium, an elliptical heterogeneous tumour (focal calcifications present) is visible. D. CT scan of the abdomen (transverse cross-section) in Case 2. The tumour shows intensive contrast enhancement.

Table 1. It illustrating tumour characteristics of the presented cases

Tumour characteristics		
	Case 1	Case 2
ENSAT Stage	II	II
Ki67	Not assessed	20%
Weiss score	Not assessed	6 points
Resection status	R0	R0
Symptoms of Cushing's syndrome	No	Yes
Mitotane serum level	14-20 mg/l (maximum mitotane serum level - 22.3 mg/l)	14-20 mg/l (maximum mitotane serum level - 29.5 mg/l)

ine device (LNG-IUD) application. The patient remained under regular gynaecological control for four years till the adjuvant mitotane therapy was completed. During this time, no meno- or metrorrhagia were observed. Hb levels were normal, and LNG-IUD tolerance was good. LNG-IUD was removed five years after its application, one year after the completion of mitotane therapy. Control TVUS and histopathological examination of endometrial biopsy performed 12 months after the removal of LNG-IUD showed endometrium within normal limits [51].

Case 2

A 30-year-old patient (Gravida 0, Parity 0) with a giant functional tumour (the presence of cortisol and androgens hypersecretion) in the right

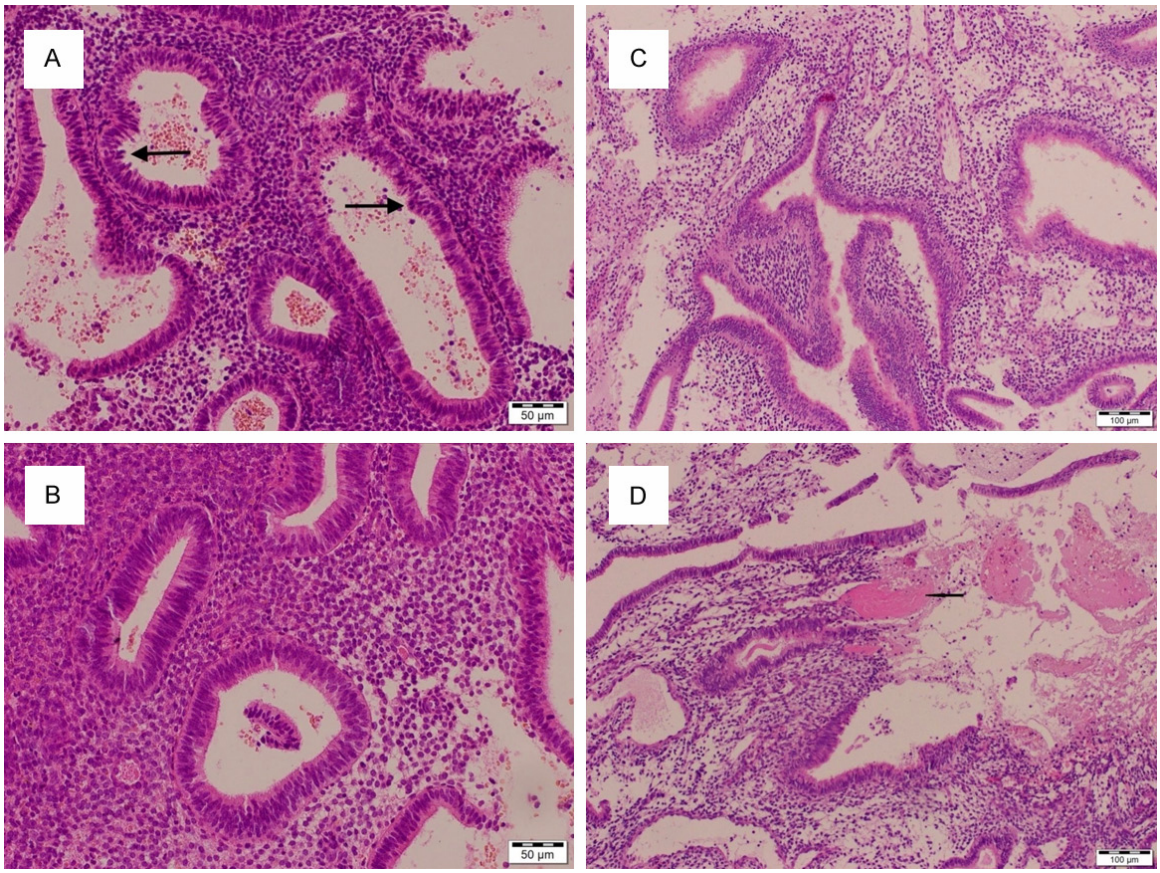

Figure 3. A, B. Hematoxylin and eosin staining (H+E) (Case 1). Endometrial (simple) hyperplasia without atypia. Irregular glands showing variation in size and shape separated by abundant stroma. Some cystic dilatation of glands is present. Nuclei are oval and pseudostratified with uniform outlines, lacking cytologic atypia. Some mitotic figures are seen (arrows). C. H+E (Case 2). Disordered proliferative phase pattern presented by slightly hyperplastic glands with branching and glandular dilatation of the lumen. D. H+E (Case 2). Signs of stromal and glandular breakdown and thrombus within vein (arrow).

Table 2. Table illustrating laboratory characteristic of the presented cases before and after of LNG-IUD insertion

Laboratory characteristics of the patients				
	Case 1		Case 2	
	Before LNG-IUD insertion	After LNG-IUD insertion	Before LNG-IUD insertion	After LNG-IUD insertion
Haemoglobin	8.9 g/dL	14.5 g/dL	9.5 g/dL	13.1 g/dL
Prothrombin time	11.5 seconds	12.9 seconds	11.8 seconds	11.1 seconds
Activated partial thromboplastin time	26.6 seconds	26.5 seconds	41.9 seconds	30.9 seconds
LH	4.80 mIU/ml	13.90 mIU/ml	<0.10 mIU/ml	20.54 mIU/ml
FSH	7.30 mIU/ml	4.02 mIU/ml	<0.30 mIU/ml	5.75 mIU/ml
Oestradiol	49,00 pg/ml	175.00 pg/dl	67,00 pg/ml	566.94 pg/ml
Testosterone	15.76 ng/dl	14.09 ng/dl	249.58 ng/dl	<7.00 ng/dl

adrenal gland (170 × 75 × 105 mm with significant enhancement in CTA) (**Figure 2C**, **2D**) underwent right-sided adrenalectomy. The patient has been treated since 2015 for schizoaffective disorder. Most likely, the overlapping symptoms of schizophrenia caused a delay in adrenal tumour diagnosis. After surgical treatment of the tumour and histopathologi-

cal diagnosis of ACC (pT2, N0, M0), the patient was qualified for adjuvant mitotane therapy with a therapeutic window of 14-20 mg/l (maximum mitotane serum level during therapy - 29.5 mg/l) (**Table 1**). After six months of therapy, the patient reported menorrhagia. In the TVUS, performed on day 8 of the cycle widened to 12.4 mm, and irregular endometrium (suggesting the presence of a polyp) was observed. The ovaries were of normal structure. The patient did not agree to a hysteroscopy. She was qualified for the endometrial pipelle biopsy [53]. Initial microscopic examination of endometrial biopsy showed small fragments of proliferative type endometrium. No signs of hyperplasia were present.

In the next four cycles, menstrual bleeding increased, Hb level after this period was 9.5 g/dL (**Table 2**). The patient did not agree to the surgical procedure and the LNG-IUD application. Continuous oral gestagen therapy was used to obtain amenorrhea, and after three months of treatment episodes of menorrhagia ended. Hb level and an ultrasound image of the endometrium were normal.

After this time, the patient, without consulting a doctor, stopped continuous oral gestagen therapy, explaining this by the lack of possibilities to see a gynaecologist. Over the next two months (12 months after the start of the adjuvant mitotane therapy), she suffered from heavy uterine bleeding during menstruation and the bleeding time increased to 10 days. The woman has been referred for a gynaecological consultation again. In the TVUS study performed on the 8th day of the menstrual cycle, a heterogeneous, almost 11 mm wide endometrium was seen. Furthermore, the presence of numerous follicular cysts within both ovaries with diameters of 24-33 mm were present. The endometrial pipelle biopsy was performed at that time. Microscopic examination revealed a disordered proliferative phase pattern presented by slightly hyperplastic glands with branching and glandular dilatation of their lumen (**Figure 3C**, **3D**), signs of stromal and glandular breakdown and thrombi within veins (**Figure 3D**). The patient was qualified for the LNG-IUD. After the insertion of LNG-IUD, she remains under gynaecological follow-up.

Discussion

In our study, we describe two cases of menstruating patients during adjuvant mitotane therapy who have come to the gynaecological department due to menorrhagia. In both patients, TVUS showed an abnormal endometrium with features of hyperplasia and the suspected presence of an endometrial polyp [51]. These features were similar for both patients included in the study. The undertaken histopathological examination revealed features of endometrial hyperplasia without signs of atypia in Case 1. In Case 2, in the first histopathological examination performed six months after the start of the adjuvant mitotane therapy, the results were not sufficient for proper diagnosis due to a small amount of endometrial tissue. The second microscopic assessment of the endometrium performed 12 months after the start of the adjuvant mitotane therapy (2 months without continuous oral gestagen therapy) revealed early signs of hyperplasia.

We may suspect that differences in the histopathological diagnoses result from the duration time of adjuvant mitotane therapy. The period of 6 months of adjuvant mitotane therapy as used in Case 2 may have been too short for fully developed morphological signs of hyperplasia. She could be at the level of functional disturbances at the time of the first endometrial pipelle biopsy. Her further clinical signs in the form of heavy menorrhagia may have been the result of at least disordered proliferating changes in the endometrium. We can also speculate that based on her clinical symptoms and a second histopathological examination, she developed some hyperplastic changes. The proper reaction to the treatment applied by us after six months and endometrial changes observed after 12 months could confirm this hypothesis.

At this stage of the study, we are unable to determine whether the effect of the mitotane treatment described above should be considered as a side effect or more as the expected after-effect of adjuvant mitotane therapy. According to the definition adopted by the World Health Organization in 1972, an adverse drug reaction is any harmful and unintended effect of a drug that occurs after administration to a person of the usual dose for prophylactic, diag-

nostic or therapeutic purposes to modify physiological functions [54].

As was mentioned in the introduction, mitotane's mode of action is not entirely explained.

This adrenal-specific agent is a derivative of insecticide dichloro-diphenyl-trichloro-ethane (DDTE), and since 1959, it has been recognised as a factor that selectively destructs synthesising glucocorticosteroids zona reticularis and fasiculata of the adrenal cortex [41].

Induction of oxidative stress caused by molecular mitotane's mode of action (due to inhibition of sterol-O-acyl-transferase one activity in the endoplasmic reticulum) seems to be the primary molecular mechanism of accumulation of toxic lipids in the adrenal cortex (free cholesterol, oxysterols, and fatty acids). Mitotane selectively shows adrenal cytotoxicity, which results in down-regulated steroidogenesis [55]. Furthermore, several additional mechanisms support the inhibitory effect of mitotane on steroidogenesis. The most important ones include the reduction of the mRNA level of two cytochromes p450 (CYP11A1 and CYP17A1), encoding proteins involved in the biosynthesis of cortisol and dehydroepiandrosterone sulphate [56].

Most likely, mitotane also affects the peripheral metabolism of steroids, affecting CBG and SHBG, increasing their concentration in the blood, and thereby reducing the level of free hormones [22, 41, 57]. Additionally, the substance directly binds to the oestrogen receptor (α) as an agonist, causing the oestrogen-like mitotane-induced effects [58].

As described previously, adverse drug side effects mainly relate to the digestive and nervous systems, allergic reactions, and coagulation disorders. These are mostly non-specific symptoms (drowsiness, fatigue, nausea, vomiting, diarrhoea, loss of appetite, headache, weakness, depression, rash, cardiac arrhythmia, weight loss, bleeding caused by prolonged bleeding time and thrombocytopenia) and are most commonly related to high doses of mitotane with a serum level above 20 mg/l, which is considered the upper limit of the therapeutic window [56, 59].

Furthermore, the specific side effects of mitotane including drug-induced encephalopathy, subacute cutaneous lupus erythematosus, dyspnoea, retinopathy and lichen planus have been described in the available literature [60-68].

While the above-mentioned side effects are associated directly with the toxic effects of mitotane, the occurrence of menorrhagia in the cases described above seems to be more complex.

Despite numerous studies on the effects of mitotane treatment on the body, knowledge about its effects on the ovaries is still insufficient. Currently, there are no recommendations regarding the use of fertility preservation procedures in women qualified for mitotane therapy. Innocenti et al. in a study performed on an animal model (mouse), showed that this substance induces a reduction in early antral follicles with a subsequent increase in secondary follicles. Ovulation disorders were also found, with a decrease in the number of oocyte ovulation, a lower number of corpora lutea and a delay in conception time in the studied models. The conclusion stated that despite the above disorders, ovarian function was maintained [69].

However, it should be noted that the knowledge about the effect of mitotane on fertility is still limited. Further research and, consequently, the creation of appropriate recommendations are required.

There are not many reports describing the effect of mitotane therapy on the hypothalamic-pituitary-ovarian axis, which disability inevitably results in disorders common in gynaecology. Basile et al. presented these disorders based on a group of 26 women. Ovarian cysts were the most common lesion (65.4%) observed an average of 8 months after initiation of the therapy. Most of these women required only close supervision without treatment. Menstrual disorders have affected 30.8% of women treated with mitotane, of which spotting occurred in 15.4%, while metrorrhagia in 7.7% of patients. There are no data regarding menorrhagia [70].

Two different studies describe ovulation disorders in the group of menstruating women in the form of ovarian cysts.

Salenave et al. in a study of 21 premenopausal women aged 18-45 years receiving mitotane in

the median starting dose of 3 g/day (range: 1.5-6 g/day) stated the appearance of bilateral ovarian cysts. Changes in the diameter of 26-90 mm occurred in 51% of cases, after a median of 11 months (range: 3-36 months) of mitotane exposure. Moreover, the authors described that the use of mitotane in this group was associated with a significant decrease in the level of androstenedione and testosterone and a significant increase in the level of luteinising hormone (LH), follicle-stimulating hormone (FSH), oestradiol (E2), also SHBG in serum [71].

Abrahamsson et al. reported the presence of benign ovarian cysts and amenorrhea in five women aged 20-45 treated for stage III-IV ACC. These patients underwent radical surgeries, including adrenalectomy, splenectomy, caval thrombectomy and then adjuvant mitotane therapy. In the conclusions, the authors stated that the synthesis of progesterone was reduced as a result of a decrease in the stimulating effect of gonadotropins and this had an impact on the formation of ovarian cysts [72]. Similar changes in Case 2 after 12 months of mitotane treatment in our study were observed.

Orisaka et al. demonstrated in an animal model (rats) that an increase in serum LH concentration increased testosterone and E2 production in preantral follicles as a result of up-regulating mRNA amount of cytochromes p450 (CYP17A1 and CYP19A1) [73].

The application of the LNG-IUD provides additional contraception. In the available literature, five cases of pregnancies during mitotane therapy have been described [74-78].

Currently, knowledge about the effects of mitotane on the human foetus is poor. The suspicion regarding the ability of the drug to cross the placenta results from the observation that an insecticide morphologically similar to mitotane (DDTE) is found in the cord blood of infants in areas exposed to its action [79]. Taking into consideration the adrenolytic activity of the drug, there is a concern for teratogenicity, although it was confirmed in only one of seven available cases. The remaining three obstetric failures were due to different reasons, and the three reported pregnancies finished with the birth of healthy newborns at term [74].

As described above, it is possible to become pregnant during mitotane therapy. That could be explained by the fact that the concentration of gonadotropins in the blood does not change significantly, and ovulation is theoretically possible [80].

In this discussion, it was hypothesized that the possible development of endometrial hyperplasia during mitotane treatment is observed after a certain period of therapy.

In the two cases described above, the therapy lasted approximately 12 months. After this time, due to the development of abnormalities in the uterine lining, pregnancy is much less likely. The cases cited above show pregnancies conceived within approximately the first year of therapy [74, 75].

When discussing the issue of contraception during mitotane treatment, it seems that it should be recommended, especially in adjuvant therapy. It is not known what form of contraception would be the best. Combined hormonal contraceptive methods (E2 and progesterone) may intensify the hypoestrogenism described above. The classic IUD is a good option, but limiting endometrial hyperplasia will be achieved with LNG-IUD.

The appearance of menorrhagia at the initial stage of therapy could be associated with the toxic effect of mitotane on the coagulation system.

The ovulation disorders, as mentioned previously, resulting in a decrease in the number of corpus luteum in the ovary during mitotane treatment, could at least partially, result in a decrease in progesterone serum level. Furthermore, SHBG (which is in increased concentration) binds free progesterone, reducing the free hormone pool in the serum.

Continuous increased E2 levels and decreased free progesterone pool during mitotane treatment could lead to relative hypoestrogenism. This would explain functional disturbances and the development of morphological changes in the endometrium. These changes are intensified by the direct effect of mitotane (as an agonist) on the oestrogen receptor (α) located in the uterine mucosa (**Figure 4**) [58].

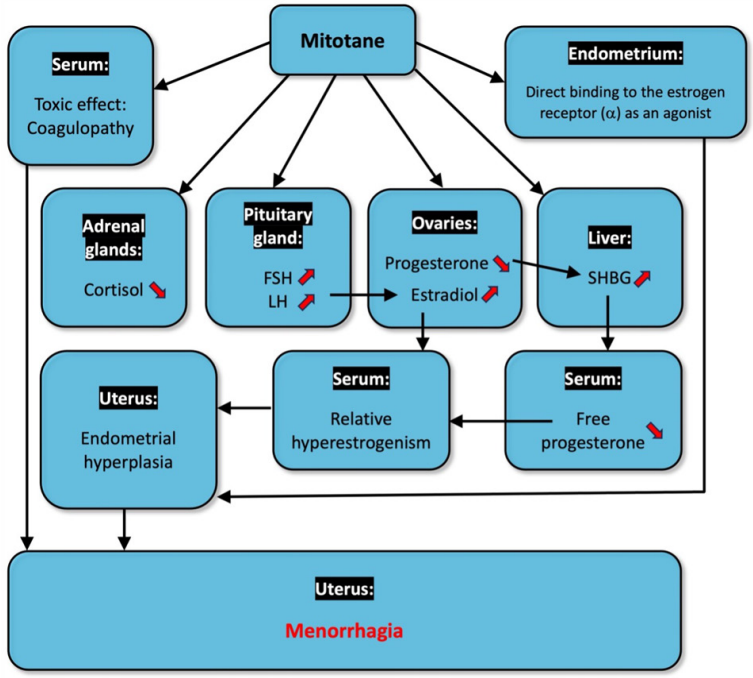

Figure 4. Diagram illustrating the effect of mitotane, leading to relative hypoestrogenism.

To summarise, menorrhagia that develops in the case of prolongated mitotane treatment should be rather treated as an expected after-effect than a side-effect of this drug. Having that in mind, women treated with mitotane for ACC should be advised about the possible appearance of metrorrhagia, and therefore constant gynaecological care might be required. Similarly, relative hypoestrogenism appears to be the expected after-effect rather than a side effect in numerous cases of gynecomastia in men and hirsutism in women during mitotane therapy [4, 10, 23, 58].

To the best of our knowledge, it is the first report of menorrhagia during ACC adjuvant therapy with mitotane.

However, the mechanism described above is not the only one that can result in abnormal uterine bleeding during the treatment of ACC. Singer et al. described a case of postmenopausal vaginal bleeding in a 63-year-old woman with metastatic ACC and high serum oestradiol levels. The author concludes that ACC may produce oestradiol in postmenopausal women, which can result in the above symptoms [81]. Therefore, during the differential diagnosis, the possibility of metastases and/or recurrences of ACC should always be taken into consideration.

We do not recommend drawing a clear conclusion regarding the effect of mitotane on endometrial functions due to the small number of cases examined and described in the literature. A multicentre trial including a representative study group, which would allow to drawing of valid conclusions is required.

Nevertheless, it should be remembered that the phenomenon of menorrhagia described here during mitotane treatment is possible and it is advisable to take this fact into account when planning treatment in menstruating women.

After over 60 years of experience with mitotane usage, knowledge about it is still insufficient, and further studies are required.

Conclusion

Based on the literature review and clinical analysis of two cases, we can draw the following conclusions: 1. Menorrhagia may be expected during adjuvant mitotane therapy of ACC in menstruating women. Heavy uterine bleeding may appear within several months since the beginning of therapy. 2. The likely mechanism for heavy menstrual bleeding is complex. Menorrhagia can occur due to the side effect of mitotane in the form of a haemorrhagic diathesis, while long-term treatment (over ten months) can lead to relative hypoestrogenism resulting in endometrial hyperplasia. 3. Continuous gestagen therapy is helpful in the treatment of the above disorders. 4. Menorrhagia during mitotane treatment may lead to severe anaemia, so this should be considered when planning treatment. 5. We do not recommend drawing a clear conclusion regarding the effect of mitotane on endometrial functions due to the small number of cases examined and described in the literature. A multicentre trial including a representative study group, which

would allow the drawing of valid conclusions is required.

Acknowledgements

The support for the study was obtained only from the scientific grant (No. 329) of the Medical University of Lublin, Poland.

Written informed consents were obtained from the patients.

Disclosure of conflict of interest

None.

Address correspondence to: Filip Szkodziak, 3rd Chair and Department of Gynaecology, Medical University of Lublin, ul. Jaczewskiego 8, 20-954 Lublin, Poland. Tel: +48-817244848; +48-505451191; ORCID: 0000-0002-2138-4694; Fax: +48-817244847; E-mail: filip.szkodziak@gmail.com

References

[1] Baudin E; Endocrine Tumor Board of Gustave Roussy. Adrenocortical carcinoma. Endocrinol Metab Clin North Am 2015; 44: 411-34.

[2] Wanis KN and Kanthan R. Diagnostic and prognostic features in adrenocortical carcinoma: a single institution case series and review of the literature. World J Surg Oncol 2015; 13: 117.

[3] Kerkhofs TM, Verhoeven RH, Van der Zwan JM, Dieleman J, Kerstens MN, Links TP, Van de Poll-Franse LV and Haak HR. Adrenocortical carcinoma: a population-based study on incidence and survival in the Netherlands since 1993. Eur J Cancer 2013; 49: 2579-86.

[4] Else T, Kim AC, Sabolch A, Raymond VM, Kandathil A, Caoili EM, Jolly S, Miller BS, Giordano TJ and Hammer GD. Adrenocortical carcinoma. Endocr Rev 2014; 35: 282-326.

[5] Guelho D, Paiva I, Vieira A and Carrilho F. Adrenocortical carcinoma: retrospective analysis of the last 22 years. Endocrinol Nutr 2016; 63: 212-9.

[6] Kebebew E, Reiff E, Duh QY, Clark OH and McMillan A. Extent of disease at presentation and outcome for adrenocortical carcinoma: have we made progress? World J Surg 2006; 30: 872-8.

[7] Nowak KM, Samsel R, Cichocki A, Ambroziak U, Roszkowska-Purska K, Łebek-Szatańska A, Koperski Ł, Otto M, Zgliczyński W and Papierska L. Prognostic factors in adrenocortical carcinoma: data from a large Polish series. Pol Arch Intern Med 2018; 128: 371-378.

[8] Ip JC, Pang TC, Glover AR, Soon P, Clarke S, Richardson A, Campbell P, Robinson BG and Sidhu SB. Improving outcomes in adrenocortical cancer: an Australian perspective. Ann Surg Oncol 2015; 22: 2309-16.

[9] Ayala-Ramirez M, Jasim S, Feng L, Ejaz S, Deniz F, Busaidy N, Waguespack SG, Naing A, Sircar K, Wood CG, Pagliaro L, Jimenez C, Vassilopoulou-Sellin R and Habra MA. Adrenocortical carcinoma: clinical outcomes and prognosis of 330 patients at a tertiary care center. Eur J Endocrinol 2013; 169: 891-899.

[10] Puglisi S, Perotti P, Cosentini D, Roca E, Basile V, Berruti A and Terzolo M. Decision-making for adrenocortical carcinoma: surgical, systemic, and endocrine management options. Expert Rev Anticancer Ther 2018; 18: 1125-1133.

[11] Libé R. Adrenocortical carcinoma (ACC): diagnosis, prognosis, and treatment. Front Cell Dev Biol 2015; 3: 45.

[12] Icard P, Goudet P, Charpenay C, Andreassian B, Carnaille B, Chapuis Y, Cougard P, Henry JF and Proye C. Adrenocortical carcinomas: surgical trends and results of a 253-patient series from the French Association of Endocrine Surgeons study group. World J Surg 2001; 25: 891-7.

[13] Erickson LA. Challenges in surgical pathology of adrenocortical tumours. Histopathology 2018; 72: 82-96.

[14] Fassnacht M, Johanssen S, Quinkler M, Bucsky P, Willenberg HS, Beuschlein F, Terzolo M, Mueller HH, Hahner S and Allolio B; German Adrenocortical Carcinoma Registry Group; European Network for the Study of Adrenal Tumors. Limited prognostic value of the 2004 International Union Against Cancer staging classification for adrenocortical carcinoma: proposal for a revised TNM classification. Cancer 2009; 115: 243-50.

[15] Erickson LA, Rivera M and Zhang J. Adrenocortical carcinoma: review and update. Adv Anat Pathol 2014; 21: 151-9.

[16] Margonis GA, Kim Y, Tran TB, Postlewait LM, Maithel SK, Wang TS, Glenn JA, Hatzaras I, Shenoy R, Phay JE, Keplinger K, Fields RC, Jin LX, Weber SM, Salem A, Sicklick JK, Gad S, Yopp AC, Mansour JC, Duh QY, Seiser N, Solorzano CC, Kiernan CM, Votanopoulos KI, Levine EA, Poultsides GA and Pawlik TM. Outcomes after resection of cortisol-secreting adrenocortical carcinoma. Am J Surg 2016; 211: 1106-13.

[17] Sada A, Asaad M, Bews KA, Thompson GB, Young WF Jr, Bancos I, Farley DR, Dy BM, Lyden ML, Habermann EB and McKenzie TJ. Comparison between functional and non-functional adrenocortical carcinoma. Surgery 2020; 167: 216-223.

[18] Duregon E, Rapa I, Votta A, Giorcelli J, Daffara F, Terzolo M, Scagliotti GV, Volante M and Papotti M. MicroRNA expression patterns in adrenocortical carcinoma variants and clinical pathologic correlations. Hum Pathol 2014; 45: 1555-62.
[19] Else T, Williams AR, Sabolch A, Jolly S, Miller BS and Hammer GD. Adjuvant therapies and patient and tumor characteristics associated with survival of adult patients with adrenocortical carcinoma. J Clin Endocrinol Metab 2014; 99: 455-61.
[20] Fassnacht M, Libé R, Kroiss M and Allolio B. Adrenocortical carcinoma: a clinician's update. Nat Rev Endocrinol 2011; 7: 323-35.
[21] Ciupińska-Kajor M, Ziaja J, Kajor M, Król R, Dobrosz Z, Konicki P, Hasse-Lazar K, Gasińska T and Cierpka L. Charakterystyka morfologiczna i kliniczna chorych na raka kory nadnercza. (Morphological and clinical characteristics of patients with adrenocortical carcinoma). Chir Pol 2006; 8: 146-55.
[22] Fassnacht M, Kroiss M and Allolio B. Update in adrenocortical carcinoma. J Clin Endocrinol Metab 2013; 98: 4551-64.
[23] Roman S. Adrenocortical carcinoma. Curr Opin Oncol 2006; 18: 36-42.
[24] Williams AR, Hammer GD and Else T. Transcutaneous biopsy of adrenocortical carcinoma is rarely helpful in diagnosis, potentially harmful, but does not affect patient outcome. Eur J Endocrinol 2014; 170: 829-35.
[25] Suman P, Calcatera N, Wang CH, Moo-Young TA, Winchester DJ and Prinz RA. Preoperative adrenal biopsy does not affect overall survival in adrenocortical carcinoma. Am J Surg 2017; 214: 748-751.
[26] Asare EA, Wang TS, Winchester DP, Mallin K, Kebebew E and Sturgeon C. A novel staging system for adrenocortical carcinoma better predicts survival in patients with stage I/II disease. Surgery 2014; 156: 1378-86.
[27] Bilimoria KY, Shen WT, Elaraj D, Bentrem DJ, Winchester DJ, Kebebew E and Sturgeon C. Adrenocortical carcinoma in the United States: treatment utilization and prognostic factors. Cancer 2008; 113: 3130-6.
[28] Dickson PV, Kim L, Yen TWF, Yang A, Grubbs EG, Patel D and Solórzano CC. Evaluation, staging, and surgical management for adrenocortical carcinoma: an update from the SSO endocrine and head and neck disease site working group. Ann Surg Oncol 2018; 25: 3460-3468.
[29] Varghese J and Habra MA. Update on adrenocortical carcinoma management and future directions. Curr Opin Endocrinol Diabetes Obes 2017; 24: 208-214.
[30] Gaujoux S and Brennan MF. Recommendation for standardized surgical management of primary adrenocortical carcinoma. Surgery 2012; 152: 123-32.
[31] Stefanidis D, Goldfarb M, Kercher KW, Hope WW, Richardson W and Fanelli RD; Society of Gastrointestinal and Endoscopic Surgeons. SAGES guidelines for minimally invasive treatment of adrenal pathology. Surg Endosc 2013; 27: 3960-80.
[32] Porpiglia F, Miller BS, Manfredi M, Fiori C and Doherty GM. A debate on laparoscopic versus open adrenalectomy for adrenocortical carcinoma. Horm Cancer 2011; 2: 372-7.
[33] Economopoulos KP, Mylonas KS, Stamou AA, Theocharidis V, Sergentanis TN, Psaltopoulou T and Richards ML. Laparoscopic versus robotic adrenalectomy: a comprehensive meta-analysis. Int J Surg 2017; 38: 95-104.
[34] Vorselaars WMCM, Postma EL, Mirallie E, Thiery J, Lustgarten M, Pasternak JD, Bellantone R, Raffaelli M, Fahey T 3rd, Vriens MR, Bresler L, Brunaud L and Zarnegar R. Hemodynamic instability during surgery for pheochromocytoma: comparing the transperitoneal and retroperitoneal approach in a multicenter analysis of 341 patients. Surgery 2018; 163: 176-182.
[35] Mirallié E, Blanchard C, Caillard C, Rodien P, Briet C, Mucci S, Drui D and Hamy A. Adrenocortical carcinoma: impact of surgical treatment. Ann Endocrinol (Paris) 2019; 80: 308-313.
[36] Maurice MJ, Bream MJ, Kim SP and Abouassaly R. Surgical quality of minimally invasive adrenalectomy for adrenocortical carcinoma: a contemporary analysis using the National Cancer Database. BJU Int 2017; 119: 436-443.
[37] Cooper AB, Habra MA, Grubbs EG, Bednarski BK, Ying AK, Perrier ND, Lee JE and Aloia TA. Does laparoscopic adrenalectomy jeopardize oncologic outcomes for patients with adrenocortical carcinoma? Surg Endosc 2013; 27: 4026-32.
[38] Miller BS, Gauger PG, Hammer GD and Doherty GM. Resection of adrenocortical carcinoma is less complete and local recurrence occurs sooner and more often after laparoscopic adrenalectomy than after open adrenalectomy. Surgery 2012; 152: 1150-7.
[39] Terzolo M, Angeli A, Fassnacht M, Daffara F, Tauchmanova L, Conton PA, Rossetto R, Buci L, Sperone P, Grossrubatscher E, Reimondo G, Bollito E, Papotti M, Saeger W, Hahner S, Koschker AC, Arvat E, Ambrosi B, Loli P, Lombardi G, Mannelli M, Bruzzi P, Mantero F, Allolio B, Dogliotti L and Berruti A. Adjuvant mitotane treatment for adrenocortical carcinoma. N Engl J Med 2007; 356: 2372-80.

[40] Berruti A, Baudin E, Gelderblom H, Haak HR, Porpiglia F, Fassnacht M and Pentheroudakis G; ESMO Guidelines Working Group. Adrenal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012; 23 Suppl 7: vii131-8.
[41] Waszut U, Szyszka P and Dworakowska D. Understanding mitotane mode of action. J Physiol Pharmacol 2017; 68: 13-26.
[42] Cremaschi V, Abate A, Cosentini D, Grisanti S, Rossini E, Laganà M, Tamburello M, Turla A, Sigala S and Berruti A. Advances in adrenocortical carcinoma pharmacotherapy: what is the current state of the art? Expert Opin Pharmacother 2022; 23: 1413-1424.
[43] Kenney L and Hughes M. Adrenocortical carcinoma: role of adjuvant and neoadjuvant therapy. Surg Oncol Clin N Am 2023; 32: 279-287.
[44] Hart MM, Reagan RL and Adamson RH. The effect of isomers of DDD on the ACTH-induced steroid output, histology and ultrastructure of the dog adrenal cortex. Toxicol Appl Pharmacol 1973; 24: 101-13.
[45] Fang VS. Cytotoxic activity of 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2-dichloroethane (mitotane) and its analogs on feminizing adrenal neoplastic cells in culture. Cancer Res 1979; 39: 139-45.
[46] Hahner S and Fassnacht M. Mitotane for adrenocortical carcinoma treatment. Curr Opin Investig Drugs 2005; 6: 386-94.
[47] Stigliano A, Cerquetti L, Borro M, Gentile G, Bucci B, Misiti S, Piergrossi P, Brunetti E, Simmaco M and Toscano V. Modulation of proteomic profile in H295R adrenocortical cell line induced by mitotane. Endocr Relat Cancer 2008; 15: 1-10.
[48] Corso CR, Acco A, Bach C, Bonatto SJR, de Figueiredo BC and de Souza LM. Pharmacological profile and effects of mitotane in adrenocortical carcinoma. Br J Clin Pharmacol 2021; 87: 2698-2710.
[49] Dang CN and Trainer P. Pharmacological management of Cushing's syndrome: an update. Arq Bras Endocrinol Metabol 2007; 51: 1339-48.
[50] Nader N, Raverot G, Emptoz-Bonneton A, Déchaud H, Bonnay M, Baudin E and Pugeat M. Mitotane has an estrogenic effect on sex hormone-binding globulin and corticosteroid-binding globulin in humans. J Clin Endocrinol Metab 2006; 91: 2165-70.
[51] Szkodziak PR, Czuczwar P, Woźniak S, Szkodziak F and Paszkowski T. Use of a levonorgestrel-releasing intrauterine device for menorrhagia treatment during adjuvant therapy of adrenocortical carcinoma with mitotane. Ginekol Pol 2017; 88: 576-577.
[52] Basile V, Puglisi S, Altieri B, Canu L, Libè R, Ceccato F, Beuschlein F, Quinkler M, Calabrese A, Perotti P, Berchialla P, Dischinger U, Megerle F, Baudin E, Bourdeau I, Lacroix A, Loli P, Berruti A, Kastelan D, Haak HR, Fassnacht M and Terzolo M. What is the optimal duration of adjuvant mitotane therapy in adrenocortical carcinoma? An unanswered question. J Pers Med 2021; 11: 269.
[53] Fu W, Zhang M, Zhou Y, Zhang S, Dong L and Wang L. Clinical study of classification and regression tree assisted endometrial biopsy in the diagnosis of endometrial carcinoma. Am J Cancer Res 2023; 13: 5394-5404.
[54] International drug monitoring: the role of national centres. Report of a WHO meeting. World Health Organ Tech Rep Ser 1972; 498: 1-25.
[55] Sbiera S, Leich E, Liebisch G, Sbiera I, Schirbel A, Wiemer L, Matysik S, Eckhardt C, Gardill F, Gehl A, Kendl S, Weigand I, Bala M, Ronchi CL, Deutschbein T, Schmitz G, Rosenwald A, Allolio B, Fassnacht M and Kroiss M. Mitotane inhibits sterol-O-Acyl transferase 1 triggering lipid-mediated endoplasmic reticulum stress and apoptosis in adrenocortical carcinoma cells. Endocrinology 2015; 156: 3895-908.
[56] Paragliola RM, Torino F, Papi G, Locantore P, Pontecorvi A and Corsello SM. Role of mitotane in adrenocortical carcinoma - review and state of the art. Eur Endocrinol 2018; 14: 62-66.
[57] Terzolo M and Berruti A. Adjunctive treatment of adrenocortical carcinoma. Curr Opin Endocrinol Diabetes Obes 2008; 15: 221-6.
[58] Rossini E, Giacopuzzi E, Gangemi F, Tamburello M, Cosentini D, Abate A, Laganà M, Berruti A, Grisanti S and Sigala S. Estrogen-like effect of mitotane explained by its agonist activity on estrogen receptor-α. Biomedicines 2021; 9: 681.
[59] Altieri B, Lalli E and Faggiano A. Mitotane treatment in adrenocortical carcinoma: mechanisms of action and predictive markers of response to therapy. Minerva Endocrinol (Torino) 2022; 47: 203-214.
[60] Pape E, Feliu C, Yéléhé-Okouma M, Colling N, Djerada Z, Gambier N, Weryha G and Scala-Bertola J. High-dose mitotane-induced encephalopathy in the treatment of adrenocortical carcinoma. Oncologist 2018; 23: 389-390.
[61] Reidy-Lagunes DL, Lung B, Untch BR, Raj N, Hrabovsky A, Kelly C, Gerst S, Katz S, Kampel L, Chou J, Gopalan A and Saltz LB. Complete responses to mitotane in metastatic adrenocortical carcinoma-a new look at an old drug. Oncologist 2017; 22: 1102-1106.
[62] Daffara F, De Francia S, Reimondo G, Zaggia B, Aroasio E, Porpiglia F, Volante M, Termine A, Di Carlo F, Dogliotti L, Angeli A, Berruti A and Ter-

zolo M. Prospective evaluation of mitotane toxicity in adrenocortical cancer patients treated adjuvantly. Endocr Relat Cancer 2008; 15: 1043-53.
[63] Maiter D, Bex M, Vroonen L, T'Sjoen G, Gil T, Banh C and Chadarevian R. Efficacy and safety of mitotane in the treatment of adrenocortical carcinoma: a retrospective study in 34 Belgian patients. Ann Endocrinol (Paris) 2016; 77: 578-585.
[64] Mayor-Ibarguren A, Roldán-Puchalt MC, Gómez-Fernández C, Albízuri-Prado F and Álvarez-Escola C. Subacute cutaneous lupus erythematosus induced by mitotane. JAMA Dermatol 2016; 152: 109-11.
[65] Pretel M, Marquès L and España A. Drug-induced lupus erythematosus. Actas Dermosifiliogr 2014; 105: 18-30.
[66] Farooq AU, Amjad W, Kochar T and Adhikari S. Mitotane-induced dyspnoea: an unusual side effect. BMJ Case Rep 2018; 2018: bcr2018225490.
[67] Vizel M and Oster MW. Ocular side effects of cancer chemotherapy. Cancer 1982; 49: 1999-2002.
[68] Schmouchkovitch A, Herry H, Thuillier P, Kerlan V, Fleuret C, Le Toux G and Boisramé S. Oral and vulvo-vaginal lichenoid reactions due to mitotane (Lysodren): a case report. Medicine (Baltimore) 2017; 96: e5075.
[69] Innocenti F, Cerquetti L, Pezzilli S, Bucci B, Toscano V, Canipari R and Stigliano A. Effect of mitotane on mouse ovarian follicle development and fertility. J Endocrinol 2017; 234: 29-39.
[70] Basile V, Puglisi S, Calabrese A, Pia A, Perotti P, Berruti A, Reimondo G and Terzolo M. Unwanted hormonal and metabolic effects of postoperative adjuvant mitotane treatment for adrenocortical cancer. Cancers (Basel) 2020; 12: 2615.
[71] Salenave S, Bernard V, Do Cao C, Guignat L, Bachelot A, Leboulleux S, Droumaguet C, Bry-Gauillard H, Pierre P, Crinière L, Santulli P, Touraine P, Chanson P, Schlumberger M, Maiter D, Baudin E and Young J. Ovarian macrocysts and gonadotrope-ovarian axis disruption in premenopausal women receiving mitotane for adrenocortical carcinoma or Cushing's disease. Eur J Endocrinol 2015; 172: 141-9.
[72] Abrahamsson G, Ekerhovd E, Janson PO, Jansson S, Ahlman H, Wängberg B and Norström A. Ovarian cyst formation in women of reproductive age receiving mitotane as part of the treatment of adrenocortical carcinoma: clinical and experimental observations. Acta Obstet Gynecol Scand 2020; 99: 1297-1302.
[73] Orisaka M, Hattori K, Fukuda S, Mizutani T, Miyamoto K, Sato T, Tsang BK, Kotsuji F and Yoshida Y. Dysregulation of ovarian follicular development in female rat: LH decreases FSH sensitivity during preantral-early antral transition. Endocrinology 2013; 154: 2870-80.
[74] Tripto-Shkolnik L, Blumenfeld Z, Bronshtein M, Salmon A and Jaffe A. Pregnancy in a patient with adrenal carcinoma treated with mitotane: a case report and review of literature. J Clin Endocrinol Metab 2013; 98: 443-7.
[75] Baszko-Błaszyk D, Ochmańska K, Waśko R and Sowiński J. Pregnancy in a patient with adrenocortical carcinoma during treatment with Mitotane - a case report. Endokrynol Pol 2011; 62: 186-8.
[76] Leiba S, Kaufman H, Winkelsberg G and Bahary CM. Pregnancy in a case of Nelson's syndrome. Acta Obstet Gynecol Scand 1978; 57: 373-5.
[77] Leiba S, Weinstein R, Shindel B, Lapidot M, Stern E, Levavi H, Rusecki Y and Abramovici A. The protracted effect of o,p'-DDD in Cushing's disease and its impact on adrenal morphogenesis of young human embryo. Ann Endocrinol (Paris) 1989; 50: 49-53.
[78] Kojori F, Cronin CM, Salamon E, Burym C and Sellers EA. Normal adrenal function in an infant following a pregnancy complicated by maternal adrenal cortical carcinoma and mitotane exposure. J Pediatr Endocrinol Metab 2011; 24: 203-4.
[79] Foster W, Chan S, Platt L and Hughes C. Detection of endocrine disrupting chemicals in samples of second trimester human amniotic fluid. J Clin Endocrinol Metab 2000; 85: 2954-7.
[80] Gentilin E, Molè D, Gagliano T, Minoia M, Ambrosio MR, Degli Uberti EC and Zatelli MC. Inhibitory effects of mitotane on viability and secretory activity in mouse gonadotroph cell lines. Reprod Toxicol 2014; 45: 71-6.
[81] Singer F. Adrenal carcinoma presenting with postmenopausal vaginal bleeding. Obstet Gynecol 1991; 78: 569-70.