Clear cell sarcoma

ABSTRACT

Clear cell sarcoma (CCS), also referred to as melanoma of soft tissues, is a rare malignant tumour of soft tissues. This tumour harbors the characteristic features of soft tissue sarcoma (STS) and is a slowly growing, painless tumour, which then acquires an aggressive course. CCS is characterized by a translocation t(12;22)(q13;q12), which in addition to the diagnostic implications may be important for targeted treatment in the future. CCS occurs mainly on the limbs, most often shin (in feet and ankle area) in the tendons and aponeurosis, often at a young age. CCS is characterized by high potential to develop metastases in regional lymph nodes (about 30% of cases). In the diagnostic process one should consider performing a sentinel node biopsy with possible subsequent radical lymphadenectomy in the case of metastases detection. Treatment of localized disease is limited to radical local excision with complementary radiotherapy. Due to the resistance to classical chemotherapy and the presence of characteristic molecular abnormalities, trials of molecular targeted therapies in this group of cancers are ongoing. In clinical trials, MET inhibitors, tyrosine kinase inhibitors (TKI) - sunitinib and pazopanib were evaluated. CCS was also one of the subtypes of tumours evaluated in the CREATE clinical trial with crizotinib.

Key words: clear cell sarcoma, crizotinib, sentinel node biopsy, sarcoma, translocation

Epidemiology

Clear cell sarcoma (CCS) is a rare type comprising approximately 1% of all soft tissue sarcomas [1]. It was first described by Enzinger in 1965 [2], and for many years in the literature it was referred to as clear cell sarcoma of tendons and aponeuroses or malignant melanoma of soft tissue. CCS is localized mainly in the lower limbs, with majority of cases localized in the area of the foot and ankle, where up to 40% of tumours are located. The upper limb is affected in 25% of cases. CCS usually develop slowly in soft tissues, adjacent to tendons, fasciae, or dissectations [3–8]. Rare CCS locations include retroperitoneal space, digestive system, bones. Cases of CCS in the kidney, torso, penis, and in the region of the head and neck or mediastinum were also described. The primary location in the skin is extremely rare with several isolated cases described so far and only one published series of cases of 12 and pooled analysis of 23 patients [9–11].

Tumours occur most often in young adults, in the 2nd–4th decade of life. CCS can develop at any age (including children and adolescents), but a small number of cases of CCS over the age of 40 years have been described [12]. It was proven that radiotherapy is a risk factor for CCS development. It is diagnosed slightly more often in women than in men [12].

Clear cell sarcomas manifest themselves as clinically silent, slow growing lesions [3, 5, 13, 14]. Tumours are often (up to 40%) manifesting as painless, although they may also cause pain, pruritus, paraesthesia, or impaired mobility of the joints [6]. The nodules can be surrounded by edema and be tender to palpation, and may also cause gait disturbances [12].

Despite slow growth and latent progression, CCS is characterized by high aggressiveness — in about 30% of patients lymph nodes or distant metastases are present at the time of diagnosis [7, 13]. Unlike most sarcomas, that metastasize via the circulatory system, approximately 50% of patients with CCS
present with metastases in the lymph nodes [5, 7, 13]. Although most frequent localization of distant dissemination of CCS is the lung, nevertheless numerous metastases to the skin, bones, liver, heart, and brain have also been described [14–16]. Generally, CCS is an aggressive tumour with a tendency of relapse, early metastasis development, and therefore short overall survival (OS) [9].

**Biology, genetics, and histopathology**

Macroscopically, CCS localize themselves usually around the tendons. They are microscopically characterized by bright, oval, or spindle-shaped cells arranged in collars separated by collagen fibres. A typical feature is the presence of giant polymorphonuclear cells with churned nuclei. CCS cells are present with abundant transparent or pale eosinophil cytoplasm and a centrally located, round nucleus with distinct nucleoli [12, 17, 18].

Because the histological and immunohistochemical features of CCS overlap with the characteristics of spindle cell melanoma and metastatic melanoma with unknown primary site (FPI, focus primarius ignotus), differential diagnosis between these units is still problematic and fraught with significant clinical consequences (Table 1) [9]. CCS exhibits characteristics of melanocyte differentiation, i.e. expression of protein A melanoma, microphthalmia transcription factor (MITF), and HMB-45 antigen, as well as the presence of melanosomes, which makes differential diagnosis of both primary and metastatic lesions difficult (Table 2) [9]. Melanin is present in CCS cells in over 70% of cases [3].

The bright cells are round or spindle-shaped, with a round central nucleus, dispersed chromatin, and a large nucleolus. The cytoplasm is light or pale-colored due to large amounts of glycogen, acid-absorbing. Intra-cellular melanin is not often visible [3, 6]. The mitotic activity of cells is small — up to 3 mitoses/10 HPF [18].

Hantschke et al. [10] indicate that it is possible to differentiate between CCS and MM (malignant melanoma) based on selected morphological characteristics (Table 1). CCS is characterized mainly by hyalinized sclerotic and reticular framework with tufts of a homogeneous population of tumour cells surrounded by delicate fibrous septa. This cell system is practically not seen in MM. What is more, CCS does not have scattered clusters of atypical melanocytes, but in most cases giant cells can be found with a characteristic rim from many peripherally located nuclei. In addition, the translocation t(12; 22) (q13; q12), observed in CCS most cases, has not been observed in MM [10, 12]. Clear cell sarcoma is a cancer that usually locates deep under the skin, with rare cases of dermal infiltration, which helps differentiate from primary melanoma, which usually also includes the epidermal layer. Differentiation from melanoma metastases is more difficult. It should be remembered that melanoma is characterized by CCS

<table>
<thead>
<tr>
<th>Immuno-histochemical staining</th>
<th>CCS</th>
<th>Melanoma</th>
<th>PEC-oma</th>
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<tbody>
<tr>
<td>Cytokeratin</td>
<td>+/−</td>
<td>+/−</td>
<td>−</td>
</tr>
<tr>
<td>S-100</td>
<td>++</td>
<td>+++</td>
<td>+/−</td>
</tr>
<tr>
<td>HMB-45</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Melan A</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosinase</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Chromogranin</td>
<td>+/−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CD68</td>
<td>−</td>
<td>+/−</td>
<td>+</td>
</tr>
<tr>
<td>Desmin</td>
<td>−</td>
<td>−</td>
<td>++</td>
</tr>
<tr>
<td>Vimentin</td>
<td>+/−</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>EMA</td>
<td>−</td>
<td>+/−</td>
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<tr>
<td>SMA</td>
<td>−</td>
<td>−</td>
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PEC-oma — perivascular epithelioid cell tumours

| Table 1. Differential diagnosis of clear cell sarcoma and malignant melanoma |
|------------------------|-----------------|----------------|
| Location               | Clear cell sarcoma | Malignant melanoma |
|                        | Deeply seated    | Primary focus on the skin |
|                        | Often associated with tendons and fascias, it does not infiltrate the dermis | Infiltration of the epidermis |
| Histopathology         | Fusiform and clear cells with small nucleoli, group of cells surrounded by partition walls fibrous | Proliferation of melanocytes in the basal layer |
| Cell polymorphism      | Small            | Big |
| Number of mitosis      | Usually small    | Often large |
| Translocation t(11; 22) | Frequent         | Absent |
| BRAF and NRAS mutations| Sporadic         | Relatively frequent |
mitotic activity, atypia, and cellular pleomorphism much higher than for sarcoma. Fibrous bands that separate sarcoma cell sockets are rare in melanoma [18]. Negative staining on epithelial markers helps distinguish sarcoma from metastases of CCS [18].

In diagnosis, the differentiation of CCS and melanoma is important (Table 1). It is helpful to detect the rearrangement of the EWSR1 gene by means of fluorescent in situ hybridization (FISH) or to determine the presence of mRNA for the EWSR1/ATF1 fusion protein by polymerase chain reaction (PCR) because they are characteristic of sarcoma and are not present in melanoma [18, 19].

After performing basic immunohistochemical staining, CCS can be indistinguishable from melanoma, and additionally we lack validated staining typical only for CCS (Tables 1, 2). Both tumours (MM and CCS) present a similar staining pattern for S100, HMB45, and melan A. This sarcoma is also often positive for tyrosinase, MITF (microphthalmia-associated transcription factor), CD117 (KIT), enolase, CD57, and vimentin. Staining against keratins membrane epithelial antigen, muscle actin, and desmin are negative [7, 17, 18, 21].

The grading of CCS, like other soft tissue sarcoma (STS), is based on the Fédération Française des Centres de Lutte Contre le Cancer (FNCLCC) system, which takes into account the degree of cell differentiation, necrosis, and mitotic activity. The CCS is characterized by a low mitotic index and a rare occurrence of necrosis, which is why most cases are classified as 1 or 2 malignancy [1, 15].

A genetic marker for CCS, which allows us to distinguish it from other cancers, is the translocation t(12; 22) (q13; q12), which is observed in more than 90% of cases [22, 23]. This translocation leads to the fusion of activating transcription factor 1 (ATF1) genes from chromosome 12q13 and Ewing sarcoma breakpoint region 1 (EWSR1) from chromosome 22q12, resulting in the EWSR1-ATF1 fusion protein that induces expression from MITF transcription factor specific for melanocytes, its interaction with another sox10 factor, and leads to cell proliferation and melanocytic dedifferentiation, which explains the similarity to melanoma [24]. Several types of fusion proteins have been documented, the most common types being 1 (fusion exon 8 EWS and 4 AFT1) and 2 (fusion exon 7 EWS and 5 AFT1) [25]. The correlation between the type of fusion protein and clinical course of the disease was not demonstrated [26]. The translocation encountered in CCS is t(2; 22) (q34; q12) leading to the formation of the EWSR1/cyclic adenosine 3.5-monophosphate response element binding protein (CREB1 protein) [22]. Both rearrangements may also occur in angiomyoid fibrous histiocytoma [27]. In addition to translocations in CCS, polsomy of chromosome 8 is often encountered [28].

Microphthalmia-associated transcription factor activation leads to the activation of c-Met, which is one of the effectors of its operation. Both cell lines were CCS and the primary tumours exhibit excessive activation of c-Met, which occurs by involving autocrine hepatocyte growth factor (HGF). C-Met expression affects increased invasiveness, chemotaxis, and survival of sarcoma cells. HGF and c-Met are potential therapeutic targets because in vitro studies have shown that blocking them with a neutralising antibody (AMG 102) or an inhibitor (SU-11274), respectively, leads to inhibition of xenografts [29]. Other receptor tyrosine kinases that can be activated in CCS are platelet-derived growth factor β (PDGFR-β) and HER3, which may also be therapeutic targets [30].

Pre-clinical studies suggest that histone deacetylase (HDAC) inhibitors may play a role in the treatment of CCS; including these inhibitors induces apoptosis, inhibiting cell growth, and decreases EWS-ATF1 expression levels in CCS cell lines. Subsequent gene expression studies also suggest other potential therapeutic targets in CCS, including fibroblast growth factor receptor 1 (FGFRI) [31].

Yang et al. [19] showed that BRAF and NRAS mutations, occurring in 51.6% and 12.9%, respectively, in melanoma, do not occur in CCS. However, Hocar et al. [15] found BRAF and NRAS mutations in two out of 22 studied cases, which implies that it does not allow to use of BRAF/NRAS mutations in the diagnosis and differentiation between melanoma and CCS.

Differential diagnosis includes malignant fibrous histiocytoma, rhabdomyosarcoma, fibrosarcoma, liposarcoma, epithelioid sarcoma, and malignant schwannoma (Table 3) [32]. CCS lesions on the limbs in histopathological diagnostics require differentiation with melanocytic tumours, clear cell myelomonocytic tumour of falciform ligament (CCMTs), paraganglioma-like dermal melanocytic tumour (CCMTD), and malignant peripheral nerve sheath tumour (MPNST), a monophasic synovial sarcoma subtype (Table 3) [33–35].

**Imaging diagnostics**

In computed tomography (CT) and magnetic resonance imaging (MRI), CCS appears as benign, well-delimited, and homogenous lesions. MRI should be performed with a gadolinium contrast. In T1-dependent images they show strong amplification, with higher signal intensity than muscles, when T2-dependent are more heterogeneous with variable signal intensity. Outbreaks of decreased signal intensity may correlate with foci of high accumulation of melanin and iron ions [28, 36, 37]. None of the radiological features allow a diagnosis based on MRI, and the final diagnosis depends on the result of histopathological examination. Positron emission tomography (PET)/CT allows detection of areas of increased metabolism and
Table 3. Differential diagnosis of clear cell sarcoma

<table>
<thead>
<tr>
<th>Malignant blue nevus</th>
<th>Clear cell sarcoma</th>
</tr>
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<tbody>
<tr>
<td>Usually located on the scalp</td>
<td>Usually located on the limbs</td>
</tr>
<tr>
<td>Superficial</td>
<td>Deep</td>
</tr>
<tr>
<td>Is often formed on the basis of benign nevus blue</td>
<td>Not associated with benign nevus blue</td>
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<thead>
<tr>
<th>Malignant peripheral nerve sheath tumour</th>
<th>Clear cell sarcoma</th>
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</thead>
<tbody>
<tr>
<td>Negative staining for melanin and HMB-45</td>
<td>Positive staining for melanin and HMB-45</td>
</tr>
<tr>
<td>Often pleomorphic</td>
<td>Rarely pleomorphic</td>
</tr>
<tr>
<td>High mitotic activity</td>
<td>Low mitotic activity</td>
</tr>
<tr>
<td>Often associated with neurofibromatosis</td>
<td></td>
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<thead>
<tr>
<th>Epithelioid leiomyosarcoma</th>
<th>Clear cell sarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular arrangement of cells and partitions</td>
<td>Usually an irregular system of cells and partitions</td>
</tr>
<tr>
<td>Negative staining on HMB-45</td>
<td>Positive staining on HMB-45</td>
</tr>
<tr>
<td>Positive staining for actin and desmin</td>
<td>Negative staining for actin and desmin</td>
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<tr>
<th>Synovial sarcoma</th>
<th>Clear cell sarcoma</th>
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<tbody>
<tr>
<td>Positive staining for cytokeratin (50–80%)</td>
<td>Negative staining for cytokeratin</td>
</tr>
<tr>
<td>Negative staining for melanin and HMB-45</td>
<td>Positive staining for melanin and HMB-45</td>
</tr>
<tr>
<td>Frequent calcification</td>
<td>Rare calcification</td>
</tr>
<tr>
<td>Translocation t(X; 18)</td>
<td>Translocation t(11; 22)</td>
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<tr>
<td>Positive staining on TLE1</td>
<td>Negative staining on TLE1</td>
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<tr>
<th>PDMT — paraganglioma-like dermal melanocytic tumour</th>
<th>Clear cell sarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Located in the skin</td>
<td>Deep location</td>
</tr>
<tr>
<td>Not very clear nuclei</td>
<td>Explicit nucleus</td>
</tr>
<tr>
<td>No necrosis</td>
<td>Necrosis often found</td>
</tr>
</tbody>
</table>

diagnostics of CCS metastases, as well as assessment of the effectiveness of surgical (and adjuvant) procedures after treatment procedures initially planned as radical [37, 38].

**Treatment of localised disease**

A common treatment in patients with CCS is surgical treatment followed by radiotherapy or adjuvant chemotherapy [12]. Neoadjuvant treatment is administered only in exceptional clinical situations, because CCS is a disease highly resistant to chemotherapy and currently there is no chemotherapy regimen (or targeted therapy or immunotherapy) providing optimal treatment in the form of tumour mass reduction [39, 40]. Single reports on the use of neoadjuvant chemotherapy indicate clinical benefit from the use of the EI scheme (epirubicin + ifosfamide) [41] and the MAID scheme (mesna 1500 mg/m²/day 1–4 + doxorubicin 15 mg/m²/day 1–3 + ifosfamide 1500 mg/m²/day 1–3 + dacarbazine 250 mg/m²/day 1–3 [31, 42, 43]. In single cases, sunitinib was used for pre-operative therapy [44]. Post-treatment follow-up for two years every three months is recommended including, among others, chest CT, due to frequent relapses in the form of metastases to the lungs [12].

**Surgery**

As with most sarcomas, a broad tumour resection is the only option for radical treatment of localized disease. The goal of surgical treatment should always be to achieve macro- and microscopically negative surgical margins, even if it requires more aggressive treatment in the form of resection of the scar after the previous surgery. This is important because resection of R1 and R2 is associated with a significantly worse prognosis [13]. It is indicated that the margin should be at least 1 cm [45]. In place of radical surgical treatment, radical radiotherapy with 70 Gy (in 2-Gy fractions) was proposed [46].

The most aggressive strategies in the form of amputations do not reduce the risk of relapse or metastasis; therefore, they should only be considered in cases where limb-sparing operations are impossible, e.g. due to infiltration of large nerve trunks [4]. Local treatment may optionally be supported by isolated limb perfusion (tumour necrosis factor-alpha + melphalan) or intra-tumoral injection (interferon-alpha), although such treatments remain the domain of clinical trials [28].

Due to the high incidence of lymph node metastases in CCS, a recently raised subject is the performance of sentinel node biopsy, which may allow earlier detection of metastases in the lymph nodes and improve the
prognosis of patients [47]. Due to the low incidence of CCS, the sentinel node biopsy data are very limited — the studies usually include only a few patients (the largest one comprised 12 patients with CCS) [48]. The percentage of positive sentinel node biopsies ranges from 30% to 50% [47–49]. The role of lymphadenectomy for locoregional control in CCS is still being evaluated [5]. Most authors suggest that it should be performed in cases where metastases in the lymph nodes were confirmed in fine needle aspiration [50, 51]. At the same time, the CCS procedure may include a sentinel node biopsy followed by radical lymphadenectomy in the case of metastases in the sentinel node [47, 52–54].

Adjuvant treatment

European Society for Medical Oncology (ESMO) guidelines recommend the use of adjuvant radiation therapy in the case of grade 2 and 3, locally advanced (> 5 cm, T2–T4), and deeply located (under the fascia) sarcomas. The majority of CCSs are formally classified as G1, hence the numerous controversies regarding adjuvant therapy. In patients with CCS, postoperative radiotherapy should be considered if it is impossible to reach R0 operating margins or if surgery is not possible due to contraindications or refusal of the patient [1]. In various retrospective studies the proportion of patients undergoing adjuvant radiotherapy was around 40% [5], but some studies did not show its effect on the improvement of OS [5]. However, in other studies such a relationship was described. On the other hand, postoperative radiotherapy improves local control of the disease, especially in the group of patients with lymph node metastases and after resection of R1 [28, 53, 55]. Irradiation can be carried out using external beams as well as brachytherapy [28]. In the case of radiotherapy, the recommended dose is 50 Gy per elective area with a dose increase to 60–66 Gy for the area of the resection bed. Neighbouring lymph node regions, in which no sarcoma metastases were found, should not be integrated into the target volume. Due to the lack of consensus, the decision on complementary radiotherapy of nodal groups with CCS metastases should be made individually for each patient. In patients from the National Cancer Centre, Tokyo the M0 patients who received adjuvant chemotherapy had better prognosis (five-year survival, 65%) than those without chemotherapy (five-year survival, 23%) (p = 0.03) [8]. Due to the high resistance of CCS to chemotherapy, its use in the adjuvant setting is not routinely recommended [1].

Treatment of metastatic disease

Palliative chemotherapy

Systemic chemotherapy is the treatment of choice for non-operative and disseminated CCSs, although CCS is a disease with a low proportion of patients responding to chemotherapy (4% partial response [PR], 37% disease stabilization [SD], progression free survival [PFS] = 11 weeks) [56]. Data on the selection of chemotherapy regimens in CCS are usually limited to retrospective analyses. The basic treatment regimens are [32]:

- doxorubicin monotherapy 60–90 mg/m²;
- doxorubicin 60 mg/m² + ifosfamide 5–9 g/m²;
- doxorubicin 60 mg/m² + cisplatin 120 mg/m².

Patients (35 cases) treated at the Istituto Nazionale dei Tumori in Milan received first-line chemotherapy with doxorubicin + dacarbazine and ifosfamide, two of whom obtained PR, three obtained SD, and six had PD according to RECIST after three months. In all cases, the clinical benefit from treatment (PR/SD) lasted less than six months [57]. The most promising data on chemotherapy was posted by a team from the Japanese Musculoskeletal Oncology Group, in which, from a group of 30 patients with CCS, a partial response was seen in 23% treated with chemotherapy, all of whom received chemotherapy with cisplatin [13]. Unfortunately, subsequent studies have not confirmed the efficacy of platinum derivatives [42], although the overall response rate (ORR) obtained after chemotherapy of M1 patients reached as much as 27% [8]. Of the 24 patients with disseminated CCS treated at the Royal Marsden Hospital and Memorial Sloan-Kettering Cancer Centre in 1990–2009, only one achieved a partial response to the chemotherapy regimen with anthracyclines. Median PFS in the analysed population was 11 weeks in the first line of treatment. Patients received anthracyclines as monotherapy or in combination with ifosfamide, platinum derivatives, or other cytotoxic agents [31, 42]. In an Italian study by Istituto Ortopedico Rizzoli, vincristine chemotherapy with cyclophosphamide and doxorubicin was used in two patients with lung metastases [7]. None of the chemotherapy regimens (doxorubicin, doxorubicin + ifosfamide, doxorubicin + dacarbazine/cyclophosphamide/vincristine) used in patients in the Dutch study from the Antoni van Leeuwenhoek Netherlands Cancer Institute in Amsterdam did bring clinical benefit [55]. Similarly, in the patients from the Hocar et al. study [15] at the Gustave Roussy Institute, doxorubicin, cyclophosphamide, platinum derivatives, dacarbazine, etoposide, ifosfamide, vincristine, interleukin 2, or interferon also showed no superiority in any of the schemes. Administration of chemotherapy with DAV (DTIC + ACNU + VCR) may provide a good response — 200 mg of DTIC, 100 mg of ACNU and 1 mg of VCR was administered intravenously on day 1. DTIC was then administered daily until the 5th day [58]. The efficacy of cisplatin [59] and temozolomide treatment (temozolomide 300 mg days 1–5/30 days) is described in patients with CCS metastases to bone [60].
Treatment in the second and subsequent lines of CCS also has a low efficiency. In a report published by the Turin team, when second-line chemotherapy was given, most patients (88%) and all patients progressed within six weeks without any clinical response; the third line of treatment was used in 30% of patients also with progression. The median survival of patients with metastatic CCS was 37 weeks [41]. In the group treated at the Royal Marsden London Hospital, 12 patients were given second-line chemotherapy, 11 (92%) had progression, and one (8%) had SD. Of the five patients treated with the third-line chemotherapy, four (80%) progressed and only one (20%) achieved SD. Finally, one patient received a fourth line of chemotherapy and maintained SD for four months. The median OS of the diagnosis was 32 months (95% confidence interval [CI] 24–39). The median OS from the onset of palliative chemotherapy was 39 weeks (95% CI 34–45 weeks) [42]. In the following lines, the treatment of patients at the Istituto Nazionale dei Tumori in Milan included the administration of high-dose ifosfamide (five patients), with one having three-month PR, another two being treated with the gemicitabine-docetaxel regimen — the first one had PR (lasting four months), and the second SD. The last of the patients was treated with trabectedin with progression [57].

Available clinical data indicate high resistance of CCS to classical cytostatic, which is also confirmed by in vitro studies. In most cases, the choice of the scheme depends on the decision of the multidisciplinary team and the centre’s own experience. The rarity of this type of STS does not allow large, multicenter, randomized trials to assess the efficacy of individual drug combinations. Single reports suggest that radiotherapy for CCS metastases may be effective at 60 Gy (fractionation at 2 Gy) [46].

Targeted treatment, immunotherapy

Due to the resistance to classical chemotherapy and the presence of characteristic molecular disorders, research on the use of molecularly targeted therapy in this group of tumours (e.g., MET inhibitors, tyrosine kinase inhibitors — sunitinib, pazopanib) is underway. Because molecular studies (described above) have shown that the development of CCS depends largely on overexpression of HGF and activation of c-Met signalling, this pathway has become an object of translational research. Early studies have shown that inhibition of Met proto-oncogene signalling (hepatocyte growth factor receptor [HGFR]) reduces the growth of CCS cells in vitro and in vivo [61]. For the phase I study with Met — tivantinib inhibitor (ARQ 197) — seven patients with CCS were included, one had a PR and two had DS [12]. In a phase 2 study (NCT00557609), both objective responses (OR) and DS were seen among 11 patients with CCS. Disease control (PR + SD) was obtained in 36% of patients, and the median duration of the response was three months [61–63]. CCS is also one of the subtypes of tumours assessed in the context of the clinical trial CREATE (EORTC 90101) with crizotinib (2 × 250 mg p.o. dosing). In this study, 26 of 28 patients with CCS had MET (+) disease, one had a confirmed PR for treatment, and 17 patients had SD. The subsequent endpoint of crizotinib efficacy in MET (+) CCS was the disease control rate (DCR), which was 69.2%. In this study the median PFS was 131 days, the median OS was 277 days, and three-, six-, 12-, and 24-months PFS were, respectively, 53.8%, 26.9%, 7.7%, and 7.7%. The authors of the study suggested that the percentage of MET (+) CCS progression-free patients treated with crizotinib is similar to the results obtained in the first line of treatment with doxorubicin in patients with metastatic STS. In further treatment lines, for patients previously treated with chemotherapy, PFS appears to be similar to that obtained with pazopanib in patients with metastatic STS [61, 64].

Only single cases of effective CCS treatment have been described so far with targeted therapy including kinase inhibitors. Mir et al. [65] published a patient with metastatic CCS metastasizing to the myocardium, in which, after application of sorafenib, a reduction in lesion size, clinical benefit was achieved in the form of pain relief and withdrawal of opioids. The time free from progression was 8.2 months [65]. In another case, the OR to treatment with sunitinib is described [66]. Sunitinib was also used at a dose of 37.5 mg/day, with a radiological, metabolic, and immunological response (loss of Melan-A/MART-1 expression on tumour cells) in the primary tumour and metastatic tumours. Sunitinib was also used in reinduction at the onset of the disease [44]. Another inhibitor of tyrosine kinases including vascular endothelial growth factor receptor type 2 and 3 (VEGFR-2/-3) studied in CCS is anlotinib, which showed PFS and OS 11 and 16 months in CCS [67].

Other potential therapeutic strategies for CCS include immunotherapy (e.g., anti-PD-1 antibodies) due to the immunophenotypical similarity of CCS to melanomas and existing examples of complete CCS response to interferon (interferon-alpha 2a) [68]. Single cases of the effectiveness of chemo-immunotherapy in CCS have also been described, including the CyVEDIC regimen in combination with ReferonA (cyclophosphamide 500 mg/m², vincristine 1.5 mg/m², epirubicin 75 mg/m², and dacarbazine 750 mg/m² i.v. q3w with interferon-alpha 2b 9,000,000 IU three times a week s.c.) [69].

The first reports on the efficacy of immunotherapy with control point inhibitors in patients with CCS have also been published. The complete response (CR) of recurrence of CCS within the chest wall was described
after pembrolizumab was used in combination with conventionally fractionated radiotherapy — a total of 50 Gy per breast and chest wall volume was added with an additional dose increase of 66 Gy per tumour volume seen in imaging studies before treatment. Treatment was well tolerated despite previous mediastinal irradiation in a nearby volume (first-grade oesophageal toxicity, second-degree skin toxicity). A significant reduction in tumour mass occurred as early as on the 10th day of irradiation, and the overall clinical response was achieved on the 18th day of radiotherapy [70]. Pembrolizumab was also reported to be effective in young patients (2 mg/kg i.v. q3w) [71]. Attempts have also been made to develop CCS vaccines. The only published study shows that this method does not seem to be effective. Metastatic tumours from CCS patients were dissected, processed, and prepared for a suspension of single CCS cells that was transduced with an adenovirus vector coding for granulocyte-macrophage colony-stimulating factor (GM-CSF), and samples were irradiated. Vaccines were administered subcutaneously and intradermally once a week for three weeks and then every other week. Although there was an increase in PD-1 expression in the tumour, there were no OR [72]. Due to extremely small cohorts of patients with CCS, they are rarely included in clinical trials, and other data on the efficacy of immunotherapy in CCS are currently not available [73].

Palliative radiotherapy

The literature describes cases of patients with unresectable CCSs, where satisfactory percentages of local disease control were obtained after palliative radiotherapy (39 Gy in fractions of 3 Gy per pelvis CCS area) [74]. Palliative radiotherapy is also applicable in relieving symptoms associated with metastatic CCS to the bones, lymph nodes, brain, or parenchymal organs.

**Survival and prognostic factors**

Five- and 10-year OS in CCS are 50–70% and 25–50%, respectively [5, 7, 13–15]. Although the local recurrence rate for this tumour was 84% in the Enzinger series, further studies observed 14–26%, and it is currently believed that about 20–55% have local recurrence [5, 15, 58]. Local recurrence may develop several years after initial diagnosis and resection [15]. Up to 40% of patients have metastases in the lymph nodes and 60% have distant metastases, mainly in the lungs [5, 15], and they usually occur within 2–4 years of diagnosis [58]. There have been reports of lung metastases 8–21 years after primary tumour resection [7]. Two-year survival in patients with lymph node or lung metastases in a series of patients at the Instituto Ortopedico Rizzoli was 40% and 0%, respectively. Five- and 10-year survival in localized disease were 72% and 53% [7], respectively, and in the Japanese population the five-year ORR was 47% (M0, 55%; M1, 20%) [8].

In multivariate analysis, significant negative prognostic factors are tumour size > 5 cm, necrosis in the tumour [9, 15], axial location [1, 7], and the presence of metastases at any time during the disease [1]. From histopathological markers only, the presence of necrosis in the tumour is negatively correlated with survival [4]. In the univariate analysis, sex (p = 0.02), tumour size (p = 0.001), localization (dermis, subcutaneous tissue, below fascia, body cavity) (p = 0.002), TNM stage (p = 0.001), and surgical margin (p = 0.04) were prognostic factors. In multi-factorial analysis, only tumour size (p = 0.02) remained an important prognostic factor [8]. In the superficial location of the tumour five-year survival rate is 80% compared to 29% for the deep location of tumour tissues. Worse results are also observed when increasing the size of the tumour. Patients with a tumour larger than 5 cm have only 28% five-year OS, while for tumour size less than 5 cm five-year survival can be expected in 71% of patients. The best prognosis was for patients with primary tumour < 2 cm [55]. In addition, as the TNM increases, the survival rate is lower. In cumulative analyses, gender also affects the survival of the patient. Women have a higher survival rate compared to men (73% compared to 36% five-year survival), although the reason for this correlation is not clear [59]. The local recurrence of the disease is not associated with worse prognosis [7], which corresponds to the theory posited by Lewis et al. [75], saying that mortality in sarcomas located on the limbs is caused by distant metastases and not local recurrence of the disease. Pre-operative duration of symptoms, mitotic index, or vascular invasion do not correlate with the survival time in these patients [16].

In the univariate analysis, among the significant negative prognostic factors, age under 30 years and male gender are also mentioned, but they are not confirmed in multivariate analyses [7]. However, it should be emphasized that in some analyses no significant prognostic factors were confirmed [17]. Discrepancies in data are primarily due to the rarity of this cancer. In the largest CCS study, covering 91 patients, the five-year OS rate was 53.8% (95% CI 41.70–64.22). For patients with initial dissemination, the median OS was 12.7 months (95% CI 10.4–21.5). Negative prognostic factors present in the univariate analysis were: male gender, period between diagnosis and metastatic stage < 24 months, metastases other than pulmonary, and no possibility of complete resection in the case of metastases. In multivariate analysis for all patients, the stage, tumour necrosis, tumour size, and localization are bad prognostic factors [76].
Clinical trials

Currently, numerous clinical trials are underway devoted to the treatment of STS patients, including CCS, comprising trials with checkpoint inhibitors and trials using checkpoint inhibitors in combination with chemotherapy or targeted therapy, using classic cytostatics or classic cytostatics in metronomic therapy, with the use of molecular-targeted drugs or vaccines, with radiotherapy, and studies using systemic therapy, including immunotherapy along with radiotherapy.

An example of a study with the use of immunotherapy in combination with molecular-targeted therapy is the ImmunoSarc study. This is a first- and second-phase study with sunitinib and nivolumab in adult patients with bone and STS (including CCS), locally advanced (unresectable) or metastatic, previously treated with at least anthracycline-based chemotherapy (NCT03277924). The completion of this study is planned for 2020.

An example of a trial using a molecularly targeted treatment is the phase 2 QUILT-3.031 trial using AMG 337, conducted in patients with CCS with a confirmed EWSR1-ATF1 fusion, advanced or metastatic (NCT03132155). The primary endpoint is response rate confirmed, whereas secondary endpoints are safety and tolerability of OS, PFS, duration of response rate and disease control. The end of this study was initially scheduled for 2020. AMG 337 is an oral MET kinase inhibitor whose safety was first evaluated in the first phase of the study in 11 patients with solid tumours [77]. In addition, based on data published in the clinical trials registry (www.clinicaltrials.gov), numerous other clinical trials are underway in STS, including CCS with different levels of advancement. Also, for some studies conducted in various solid tumours, inclusion of CCS patients was planned. Detailed information on the clinical trials conducted in STS are included in the article entitled “Advances in systemic treatment of advanced soft tissue sarcomas”.

Summary and conclusions

Due to the risk of late relapse, long follow-up periods are necessary, primarily to detect local recurrence at an early stage where local treatment can still be provided with a good therapeutic effect. However, patients who experience recurrence of the metastatic form of the disease die from the disease, and close observation, unfortunately, does not alter the natural course of the disease in these patients [1].

References


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361


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