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Cervical lymph node metastases of squamous cell carcinoma from an unknown primary site: a favourable prognosis subset of patients with CUP

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Abstract Squamous cervical cancer of unknown primary site (SQCCUP) presents in patients as neck lymph nodes involved by squamous carcinoma in the absence of identifiable primary in the head, neck or lung. This CUP subset affects male patients previously exposed to alcohol and tobacco, though a proportion of cases may be related to chronic infection of the oropharynx by human papilloma virus. A standardised diagnostic work-up consisting of panendoscopy of the upper aerodigestive tract, CT of the chest/abdomen and histology supplemented by immunohistochemistry is warranted for the diagnosis. The scant available evidence on the molecular biology of the disease is reviewed. The cornerstones of management are excisional biopsy or surgical extirpation of the disease followed by bilateral neck external beam radiotherapy and chemotherapy. The necessity for complete surgical resection of involved neck nodes, irradiation of all head/neck mucosal sites and administration of concurrent chemotherapy is currently being debated. Aggressive multimodal therapy results in long-term disease control in 50–60% of patients, though data are mainly based on retrospective cohorts. Factors predicting for superior patient outcome are radical management with surgery or radiotherapy, low stage and volume of disease, absence of extracapsular spread and good performance status. Recently introduced molecular profiling platforms may provide biological classification to a primary tissue of origin as well as insights into the pathophysiology of this clinical entity.

Keywords Cancer of unknown primary · Cervical metastases · Squamous cell carcinoma

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Epidemiology

Cancer of unknown primary site (CUP) represents a heterogenous group of malignancies presenting with distant metastases without an identified primary tumour at diagnosis [1]. As a clinical entity defined by the exclusion of the presence of a primary tumour, the standardised work-up necessary for investigation of a primary is pivotal: thorough physical examination including breasts, skin, genitals and pelvis, thoracoabdominopelvic CT, mammography and a battery of routine haematologic/biochemical tests are required. Additional work-up will be directed by symptoms, signs and laboratory test abnormalities. Determination of serum PSA, AFP and HCG is also warranted in order to pick up occult prostate or germ cell tumours. However, even after application of rigorous diagnostic work-up, the primary tumour will not be diagnosed in 3–5%of patients with malignancies. CUP consists of distinct clinicopatho-

logic subsets. The vast majority of patients (80%) harbour systemic visceral metastases of adenocarcinoma or poorly differentiated carcinoma and belong to the poor risk CUP subset. Only 20% of patients belong to favourable prognosis subsets (serous papillary peritoneal carcinomatosis, adenocarcinoma in axillary lymph nodes, squamous lymphadenopathy of the neck or inguinal regions, blastic bone metastases with PSA expression). These favourable CUP subsets are characterised by biological behaviour similar to an occult primary tumour in the relevant region (peritoneum, breast, head and neck, genitals, prostate) and patients frequently enjoy long-term survival. Squamous cell carcinoma affecting cervical lymph nodes in the absence of extracervical metastases, squamous cervical cancer of unknown primary site (SQCCUP), belongs to the favourable group of CUP patients.

In 1957, the first definition of cervical lymph node metastasis of an unknown primary site was reported by Comess et al. [2]. Cervical lymph node metastases from an unknown primary site of squamous cell histology constitute approximately 5% (range, 1–10%) of all head and neck cancers [3]. The annual incidence of SQCCUP tumours is 0.34 cases per 100,000 per year [4]. Median age is around 57–60 years (range 30–80 years) and almost 80% of the patients are males. They usually have a history of chronic tobacco or alcohol use. Over the last three decades a well documented rise in the incidence of oropharyngeal squamous carcinomas has been linked to chronic infection of the oral mucosa by human papilloma virus (HPV) serotypes 16 and 18 [5]. In comparison to patients harbouring tobacco- or alcohol-related head/neck squamous cancer, patients with HPV-associated oropharyngeal cancers are younger by five years, non-smokers, non-drinkers, respond better to radiotherapy and survive longer. The impact of this new form of head/neck squamous cancer on the epidemiology, incidence and prognosis of squamous cervical lymph node metastases from unknown primary is unclear at present. The detection of HPV DNA or RNA by PCR in lymph nodes involved by SQCCUP offers promise for detection of occult primaries in the oropharynx, as this is the most likely site of chronic HPV infection leading to carcinogenesis.

Squamous cell histology is the most common type, representing 75% of cases, followed by undifferentiated carcinoma and adenocarcinoma. Regarding the distribution of involved cervical lymph nodes, jugulodigastric nodes are the most commonly affected (71%), followed by midjugular nodes (22%) [6]. In this paper only patients with squamous cell histotype will be discussed, as patients with other histological types are managed differently and have a different prognosis.

Molecular biology

Scant efforts have been invested in unravelling the molecular biology of unknown primary cancer. A major problem with any research effort is the heterogeneity of this clinical entity, which would result in multiple molecular aberrations being responsible for the distinct behaviour of the different CUP subsets. The molecular biology of SQCCUP has not been investigated, probably due to the rarity of this patient population, and any insights into its pathophysiology stem from extrapolated data from head/neck cancer of known primary. Chromosomal abnormalities commonly seen in head/neck carcinomas are 3p, 9p deletions and loss of heterozygosity at 4q, 8p, 11q, 13q, 14q and 17p. Califano et al. studied microsatellite markers by PCR in 18 SQCCUP and reported loss of heterozygosity in 3p, 9p, 11q and 17p [7]. Common genetic alterations in head/neck cancer of known primary (incidence 30–60%), such as COX2 activation, EGFR overexpression and inactivation of the tumour suppressors p16, p21, p53 and pRB, have not been investigated in SQCCUP. Overexpression of MET and activation of cyclin D1, Ras and AKT occur in one third to one quarter of head/neck cancers, resulting in increased cellular proliferation, apoptosis inhibition, invasion and metastasis [8]. Angiogenesis is particularly active in head/neck cancer and microvessel density and VEGF expression by immunohistochemistry or PCR have been associated with aggressive disease course and poor outcome [9].

Some data have recently been reported on molecular aberrations in unselected populations of patients with CUP tumours [10, 11]. Immunohistochemical Ras oncoprotein was overexpressed in a third of 26 CUP cases and HER2 in 10–20% in four small series. EGFR and COX2 oncoproteins were overexpressed in 12–60% of CUP tumours, although no activating EGFR gene mutations or gene amplification were found in 50 CUP cases examined. Similarly, despite overexpression of c-KIT and PDGFR in 11–18% of CUP cases, no activating mutations of the relevant genes were identified in 50 CUP tumours. Immunohistochemical overexpression of the antiapoptotic protein BCL2 was reported in 40% of 40 CUP tumours and p53 protein overexpression in approximately half of CUP cases examined in several series. The frequency of p53 gene mutations was found to be 26% in 15 CUP and eight CUP cell lines. Active angiogenesis was evident in CUP: CD34-based assays of microvessel density and immunohistochemical VEGF-A expression disclosed increased angiogenic activity in 26–83% of examined CUP tumours in several retrospective series of moderate sample size. However, the exact incidence and biologic significance of the molecular traits described above in SQCCUP is unknown and their potential for targeting with smart drugs unproven.

Diagnostic evaluation

The diagnostic approaches in patients with SQCCUP refer first to the establishment of the histopathological type of the tumour and secondly to the detection of the primary tumour site. Therefore, the diagnostic manoeuvres include

Table 1 Location of neck nodes and possible site of primary tumour

Level	Neck nodes involved	Possible primaries
I	Submandibular nodes	Mouth floor, lips, anterior tongue
II	Jugulodigastric/upper jugular nodes	Epipharynx, base of tongue, tonsils, nasopharynx, larynx
III	Middle jugular nodes	Supraglottic larynx, inferior pyriform sinus, post-cricoid region
IV	Inferior jugular nodes	Hypopharynx, subglottic larynx, thyroid, oesophagus
V	Supraclavicular	Lungs, thyroid, breast, gastrointestinal system

physical examination, fine-needle aspiration (FNA) or biopsies, imaging studies and endoscopy.

Physical examination

A painless and unilateral cervical mass is the most common clinical presentation. The site of palpable cervical lymphadenopathy could be useful in suggesting the possible primary tumour site. In patients with squamous cell histotype the jugulodigastric and midjugular lymph nodes are most commonly involved, whereas metastatic adenocarcinoma is more frequently diagnosed in the low cervical or supraclavicular areas.

In addition, based on the metastatic lymph node level, several probable sites of the primary tumours can be predicted, i.e., (a) if submandibular nodes (level I) are involved the primary site could be in the floor of the mouth, lips and anterior tongue; (b) if jugulodigastric or upper jugular nodes (level II) are affected, search for a primary tumour in epipharynx, base of the tongue, tonsils, nasopharynx and larynx, (c) if middle and lower jugular nodes (levels III and IV) are involved, the most likely primaries are located in hypopharynx or larynx; and (d) if supraclavicular nodes (level V) are the metastatic sites, the possible primary tumours could be derived from the lungs, thyroid, breast, gastrointestinal or genitourinary system [7, 8] (Table 1). The most commonly involved level is level II (30–50%), followed by levels I and III (10–20%) and levels IV and V (5–10%).

Cytology and histopathology

FNA is most commonly used as a first-step diagnostic procedure to establish malignancy. The diagnostic accuracy of FNA in these patients is closed to 95% [12–14]. Incisional biopsy of enlarged cervical nodes remains controversial since higher rates of local recurrence have been observed due to seeding of tumour cells along the tract. However, open biopsy is indicated if the mass is suspected to be lymphoma, sarcoma, melanoma or adenocarcinoma [15, 16]. While traditional histochemistry has been established as a useful technique in other tumour types, it has not proven particularly helpful in the diagnostic work-up of SQCCUP. Advanced molecular techniques such as *in situ* hybridisation or polymerase chain reaction could be useful in detecting Epstein-Barr virus (EBV) or HPV, differentiating a nasopharyngeal or oropharyngeal primary cancer, respectively [17, 18]. Some investigators have controversially advised diagnostic tonsillectomy in the presence of HPV DNA in involved cervical lymph nodes.

Imaging studies and endoscopy

If history, physical examination and imaging studies are unrevealing to identify a primary site, the patient should undergo a panendoscopy under anaesthesia with the use of a flexible nasopharyngoscope. Blind biopsies from nasopharynx, tongue base, tonsil and pyriform sinus are recommended. Oesophagoscopy and bronchoscopy are also parts of the panendoscopic examination [12, 19].

Imaging investigation in SQCCUP patients includes computed tomography (CT) scan, magnetic resonance imaging (MRI) and positron emission tomography (PET). The goals of performing imaging studies in these patients include, first, the detection of the primary site in the head-neck region or in the lungs and, second, the staging evaluation of lymph nodes status before any locoregional treatment. Imaging should be performed prior to any invasive procedure or treatment in order to avoid any diagnostic misinterpretation. CT scan is considered as the imaging study of choice, because it has a low cost and offers detailed anatomical information. Primary tumour detection rate is approximately 22% [20, 21]. MRI has a higher accuracy in identifying the primary site – 36%. Due to better soft-tissue definition comparing to CT scan, it is more useful for investigating the area of nasopharynx and oopharynx [22, 23].

PET has also been used in patients with SQCCUP [24–26]. A review of 16 studies using ^{18}F-FDG PET showed a diagnostic accuracy in detecting the primary of 24.5%, while sensitivity and specificity were 88% and 75%, respectively. Delgado-Bolton et al. performed a meta-analysis of the performance of ^{18}F-FDG PET in primary tumour detection in 15 CUP studies and reported a sensitivity of 87% and specificity of 71%. In seven of those studies, PET investigations were applied in patients with SQCCUP. Unidentified metastases were found in cervical lymph nodes (67% of patients) and brain (14%), while the primary tumour was identified in a third of cases (range 21–52%), most commonly in the lung or oropharynx [27].

A disadvantage of FDG-PET, however, is its lack of anatomic information with precise localisation of FDG accumulation. Therefore, the application of combined FDG-

PET/CT or MRI could offer greater value for the detection of primary site. Several studies with FDG-PET/CT demonstrated identification of primary tumour in 48–73% of the cases and modification of treatment plans in almost 30% of the patients [28]. Kwee and Kwee recently published a meta-analysis of 11 studies enrolling 433 CUP patients who underwent PET/CT [29]. The overall primary tumour detection rate was 37%, sensitivity 84% and specificity 84%. In four studies, patients with SQCCUP only were enrolled, among whom the primary tumour detection rate varied from 28% to 57%. Sensitivity and specificity were in the ranges 70–100% and 73–100% respectively. The identified primaries were most commonly found in the lung (33% of primaries) and in the oropharynx (16%), pancreas (5%) and breast (4%). In three trials comparing the utility of PET/CT vs. PET, the authors reported that although PET/CT identified a few more primaries than PET, these differences were not statistically significant. Overall, PET/CT modified therapy in 20–60% of cases.

Prognostic factors

The prognostic outcome of patients with SQCCUP is based on several endpoints such as the overall survival, disease-free survival, distant failure and locoregional control. Numerous treatment-, patient- or tumour-related variables have been implicated. However, the most prominent prognostic factors correlated with disease outcome are two tumour-related variables, the lymph nodal stage and the extracapsular spread. Jereczek-Fossa et al. reviewed published prognostic information in patients with SQCCUP and reported negative margin surgical resection of neck nodes, surgical extirpation of nodal disease followed by neck radiotherapy, bilateral irradiation of the neck and head/neck mucosa, avoidance of delays in radiotherapy and treatment after 1990 as the most important treatment-related variables predicting for superior patient outcome [3]. Among patient- or tumour-related variables, good performance status, young age, absence of weight loss, low volume nodal disease in the neck, absence of fixation or of low cervical adenopathy, no extracapsular extension, and low grade were favourable prognostic factors. In two recent papers by Beldi et al. and Huang et al., the prognostic importance of bilateral irradiation of neck and mucosa, low nodal stage, absence of extracapsular extension and curative treatment (surgery and/or irradiation) were confirmed in retrospective series of a total of 161 patients with SQCCUP [30, 31]. Table 2 demonstrates the neck nodal staging.

Table 2 Nodal staging in patients with SQ-CUP

Nodal disease	Nodal characteristics
N1	Single ipsilateral node <3 cm
N2a	Single ipsilateral node 3–6 cm
N2b	Multiple ipsilateral nodes <6 cm
N2c	Bilateral or contralateral nodes <6 cm
N3	Lymph node >6 cm

Treatment

The optimal therapeutic management of patients with SQ-CUP remains controversial as a result of the absence of randomised studies comparing treatment options. Therefore, the treatment is mainly based on non-randomised evidence as well as on institutional policies.

Surgery

Surgical therapy includes excisional biopsy, neck dissection ("radical", "modified" or "selective") and tonsillectomy. "Radical neck dissection" refers to the removal of the levels I–V neck nodes, which at the same time sacrifices the spinal accessory nerve, internal jugular vein and sternocleidomastoid muscle. "Modified radical neck dissection" removes the same nodal levels but spares the rest of the neck structures. It is important to notice though that preservation of spinal accessory nerve saves shoulder mobility. "Selective neck dissection" targets specific nodal groups and it is considered as the safest operational procedure.

Patients with N1 or N2a limited disease without extracapsular extension could be treated with surgery alone. Locoregional control rates range from 80% to 90%, median nodal recurrence rate is about 34% and 5-year overall survival rate up to 65% [32–34]. Therefore, neck dissection alone is advocated only for patients with N1 and N2a disease without extracapsular spread, whereas postoperative irradiation is indicated in cases with a history of incisional or excisional biopsy and in patients with extracapsular extension [35–42].

Tonsils are considered one of the most common sites of a hidden primary site in patients with SQ-CUP. Although the true incidence is not known, it is estimated to be between 18 and 40%. Tonsils have been incriminated as the occult primaries especially in patients bearing head and neck tumours associated with chronic HPV infection of the upper aerodigestive mucosa. Various reports suggest that directed random biopsies, and unilateral or bilateral tonsillectomy should be part of the screening for detection of the occult primary tumour [43, 44]. It is interesting that in 10% of the cases the primary tonsular lesion is located contralateral to the metastatic cervical nodes. Nowadays, several specialised centres recommend bilateral tonsillectomy (screening tonsillectomy) as standard procedure in the investigation of patients presenting with subdigastric, mid-jugulocarotid or submandibular nodal metastases.

Table 3 Occult primary sites to be included in radiotherapy fields, according to the level of the enlarged lymph nodes

Level of the neck	Sites to be irradiated
I	Oral cavity, Waldayer's ring, oropharynx, both sides of the neck. Protection of larynx
II, III, (upper) V	Nasopharynx, oropharynx, hypopharynx, larynx, both sides of the neck, to the level of the clavicles
IV only	Waldayer's ring, larynx, hypopharynx, both sides of the neck
Lower level V	Larynx, hypopharynx, both sides of the neck, generous regional portal to include adjacent apex of the axilla
Preauricular	Radiotherapy alone (or combined with parotidectomy). Squamous cell carcinoma is suggestive of skin cancer

Irradiation

The most frequently used therapeutic approach by the majority of centres consists of surgical removal of the neck disease followed by postoperative radiotherapy (or radiochemotherapy) either to the neck, or to both the neck and the potentially involved mucosa. Indications for postoperative radiotherapy in SQCCUP patients are excisional or incisional biopsy of the neck before definitive treatment, extracapsular extension of the tumour and multiple positive lymph nodes (stage N2b or higher). However, primary radiotherapy or chemoradiotherapy (in fit patients) may be given in patients with initial stage N2b or N3 disease as a sole treatment or followed by neck dissection 4–6 weeks later. Patients with large nodes fixed to the adjacent structures (e.g., to the carotid sheath) and patients with a low performance status and comorbidities may also be treated by primary irradiation.

Although the value of irradiation of the potentially (occult) primary sites has not been confirmed by randomised studies, many authors have observed that mucosal irradiation reduced both the emergence of primary tumour and regional recurrence, but without affecting overall survival [32, 33, 35–37]. In a recent study, a higher 5-year overall survival rate has been reported for patients treated with extensive radiotherapy, including neck nodes and pharyngeal mucosa, relative to those treated by more limited volumes (57.6% vs. 24%, $p<0.01$). Radiotherapy portals should encompass the sites shown in Table 3, according to the level of the neck affected. The dose usually given with standard fractionation (dose per fraction of 1.8–2 Gy) is 65–70 Gy to the involved nodal stations and 50 Gy for the uninvolved neck and mucosal sites. In case of clinically suspicious mucosal sites, a dose of 60–64 Gy is recommended (Table 3).

The use of three-dimensional (3D) radiotherapy techniques is suggested to have an impact on survival [30], although there may be a selection bias to this conclusion: most patients who received 2D radiotherapy were treated 20 or more years ago when diagnostic procedures were less sophisticated. Therefore patients with distant metastases at the time of initial treatment were more frequently included in those studies.

The main acute radiation toxicity consists of dysphagia and mucositis, especially in patients treated with combined radiochemotherapy compared with those treated with radiotherapy alone. Xerostomia is the main late complication of radiotherapy. Other late effects are persisting oedema of the larynx or skin, soft tissue fibrosis, necrosis and osteoradionecrosis [32–38]. Huang et al. reported severe (grade 3–4) acute mucositis in 34% of patients, insertion of a nasogastric feeding tube in 54%, and severe late xerostomia or neck fibrosis in 7% [31]. Combined with postoperative complications and post-chemotherapy toxicity, antineoplastic treatment can potentially affect the quality of life especially of the long-term surviving patients. This underlines the significance of advanced radiotherapy techniques, such as 3D conformal and IMRT, regardless of any anticipated benefit on tumour control.

Combined modality approaches

Concurrent chemoradiotherapy in patients with locally advanced squamous cell carcinoma of the head and neck significantly improves response rate and overall survival [45–50]. In addition, the combination of platinum-based chemotherapy with cetuximab increased efficacy as firstline treatment in patients with recurrent or metastatic head and neck cancer [49]. All these studies are large well conducted randomised studies published during the last few years.

Unfortunately, up to now there have been no randomised reports on the efficacy of chemoradiotherapy in patients with SQCCUP. To the best of our knowledge there are only 4 retrospective studies with approximately 100 patients treated with various cytotoxic drugs (platinum or non-platinum). Chemotherapy was administered before, during or after radiotherapy and results in some studies were compared with historical controls [30, 50–52].

In the oldest study De Braud et al. reported on 16 SQCCUP patients treated with chemotherapy before, during or after radiotherapy [50]. Complete response rate to combined treatment was 81% and median survival was 24 months. The median survival was 37 months in the chemoradiotherapy group vs. 25 months in the surgery and radiotherapy groups. Significant limitations of this retrospective study are the heterogeneity in chemotherapy regimens and schedule administered as well as in irradiation treatment fields and dose.

In the second study, Argiris et al. treated 25 patients in five sequential prospective phase II trials with standardised split-course radiotherapy with concurrent 5FU and hydroxyurea (FHX regimen) [51]. In two trials a third drug was added, either cisplatin or paclitaxel. A median total dose of 60 Gy was administered to potential sites of

Table 4 Outcome of SQCCUP patients managed with combinations of surgery, irradiation and chemotherapy

Investigators	Number of patients	Therapy	N2/N3 status (%)	5-year survival
Jesse 1973	184	S	45/38	All patients, 53%
		RT	75/36	
		S+RT	55/31	
Colletier 1988	136	S + RT	78/13	60%
De Braud 1989	41	S and/or RT, CRT in 16 pts	39/56	Median OS 24 months
Marcial-Vega 1990	72	RT or S+RT	54/20	45%
Harper 1990	69	RT or S+RT	NR	66%
Maulard 1992	113	S+RT	40/19	38%
Reddy 1997	52	RT or S+RT	71/29	51%
Grau 2000	277	S and/or RT	49/34	36%
Friesland 2001	51	RT or S+RT	55/30	41%
Argiris 2003	25	CRT or S+CRT	76/24	75%
Shehadeh 2006	37	S+CRT	58/22	1-year OS 95%

mucosal primaries and the neck bilaterally. The 5-year progression-free and overall survival rate was 87% and 75% respectively. Severe acute mucositis occurred in 68% of patients, while a nasogastric feeding tube was inserted in 56% of them. Patients with N3 or supraclavicular lymphadenopathy had markedly worse outcome (3-year overall survival 33%).

Beldi et al. retrospectively reviewed management and outcome data on 113 SQCCUP patients, the largest series reported to date [30]. Half of all patients received radical surgery followed by radiotherapy, while 48% received radiotherapy only. Irradiation of the head/neck mucosa and bilateral sides of the neck was applied in 67 patients, while 45 were irradiated at the neck only (ipsilaterally or bilaterally). The median total dose of ionising radiation administered was 60 Gy. The main side effects were mucositis, dysphagia and xerostomia, with severe late effects observed in less than 7% of patients. Only 18% of the patients received chemotherapy. The 5-year survival was 41%, with the primary tumour manifesting in 20% of patients. Superior survival was seen in patients managed radically with extensive irradiation.

In the most recent retrospective report by Shehadeh et al., 37 patients with SQCCUP were managed with neck dissection followed by concurrent high-dose cisplatin and bilateral neck/mucosal sites irradiation [52]. A total dose of 64 Gy was administered in the involved side of the neck and 50 Gy to uninvolved neck and mucosal sites. At a median follow-up of 42 months, 89% of patients were alive. Only two regional and four distant relapses were observed. These encouraging results were coupled to safety of therapy (incidence of severe mucositis 46%, xerostomia 30%). Based on these encouraging preliminary results, prospective multicentric studies in a larger number of SQCCUP patients will be warranted, in order to establish the efficacy of concurrent chemoradiotherapy in a cohort of patients with bulky neck disease. The outcome of SQCCUP patients managed with combinations of surgery, irradiation and chemotherapy is summarised in Table 4.

Recurrent disease

Despite radical therapy with surgery, radiotherapy or chemoradiotherapy, more than 40–50% of patients with SQCCUP will eventually relapse either in the head/neck or systemically. Long-term disease control is feasible for less than 20% of relapsing patients. Surgical extirpation of recurrent disease and/or re-irradiation to doses >54 Gy are therapeutic modalities associated with cure in selected patients [53]. Still, the majority of relapsing patients will harbour locoregional disease that cannot be treated radically or distant metastases. For these patients, any therapy is purely palliative, aiming for symptom control, preservation of quality of life and modest survival prolongation. Chemotherapy is the mainstay of treatment in such circumstances and data on its efficacy are extrapolated from patients with overt head/neck cancer. Standard regimens are platinum compounds combined with 5-fluorouracil infused over 4–5 days. Recently, the addition of cetuximab, an anti-EGFR monoclonal antibody, to cisplatin/5FU chemotherapy significantly prolonged overall survival (median survival 10.1 vs. 7.4 months, p=0.04) of 442 patients with relapsed or metastatic EGFR-expressing head/neck squamous cancer in the context of a prospective phase III trial [49]. For patients previously exposed to platinum compounds, weekly methotrexate or single-agent taxanes, liposomal doxorubicin and bleomycin have shown limited activity in small phase II trials [54].

Discovery of primary site and molecular platforms

The incidence of the appearance of primary site in patients with SQCCUP is around 10% (ranging between 5% and 30%) and it usually occurs within the first 2 years of treatment. Several authors consider primary tumours arising later than 5 years after primary diagnosis as second primaries. The most common sites of the appearance of primary tumours include nasopharynx, base of the tongue, tonsil

Table 5 Gene profiling platforms used for biological classification of SQCCUP

Investigator	Array	Number of genes	CUP number	Occult head/neck squamous tumour
Van Laar	cDNA	495	>500	Less than 5%
Tothill	cDNA	79	13	Less than 10%
Talantov	cDNA	10	48	Less than 10%
Hainsworth	cDNA	10	69	Less than 5%

Table 6 Primary tumour identification and CUP patient outcome in time

Decade	Patient number	No. of studies	Mean 5-year survival (range)	Appearance of primary
1960–69	256	3	26.5% (16–34)	–
1970–79	1084	9	29% (9–54)	18%
1980–89	449	4	40% (21–60)	16.5%
1990–99	1235	13	49% (27–60)	10.5%
2000–08	1113	12	55% (36–79)	11%

and pyriform sinus. Patients undergoing bilateral tonsillectomy have a 3-fold increased chance of discovering the primary site in the tonsils [55]. On the contrary, treatment with radiotherapy bilaterally to the neck as well as to mucosa sites seems to considerably decrease the appearance of mucosal primary sites, suggesting early eradication of the occult malignant clone [56].

High-throughput molecular profiling technologies have provided rapidly accumulating data on expression (transcription) of multiple genes in several human tumours. These oligonucleotide hybridisation microarray platforms, coupled to bioinformatic analysis tools, enable scientists to identify a 'typical' multigene expression profile for each solid tumour. Accordingly, gene expression in CUP tumours may be studied by the use of the same methods and compared to the 'typical' genetic profiles of known cancers. Recently published data show that a primary tissue of origin can be molecularly assigned with high accuracy in 70–85% of CUP cases [57]. Four studies reported use of multiple gene expression profiling platforms for the identification of tissue of origin in CUP. In these studies, three microarray genetic signatures biologically classified more than 500 CUP cases with 76–96% accuracy (Table 5). The CUPPrint chip (Agendia, Amsterdam) used 495 genes, whereas data on the other two multigene expression chips were used for the generation of low-density RT-PCR arrays with 79 and 10 genes, respectively. Breast adenocarcinoma was the most common primary tumour of origin of CUP cases, followed by pancreatic, colorectal, lung, hepatobiliary, renal, bladder, ovarian and gastric cancer. A head/neck squamous primary was biologically assigned as the occult primary in less than 5–10% of cases analysed.

Despite the accuracy of microarrays in identifying molecular similarities, it is unknown if a squamous head/neck CUP molecularly assigned to a head/neck primary tumour indeed behaves similarly to a typical metastatic tumour of the same primary [58]. Head/neck CUP tumours and metastatic head/neck tumours of overt primaries may harbour some distinct genetic or epigenetic lesions. The hope that molecular classification of CUP followed by primary site-specific therapy would improve patient outcome should be validated in prospective clinical trials. In such a trial, a poor-risk CUP patient cohort should be randomised to empiric management or molecularly assigned primary site-specific management.

Conclusions

Squamous carcinoma of head/neck lymph nodes constitutes a clinicopathologic subset of CUP affecting male patients older than 50–60 years, commonly exposed to alcohol and tobacco. Imaging of the chest/abdomen and thorough panendoscopic examination of the upper aerodigestive tract are mandatory for the exclusion of an occult primary. Available data suggest that the natural history of the disease and response to therapy are similar to that of metastatic head/neck squamous cell carcinoma. Indeed, 10–20% of SQCCUP patients eventually manifest an overt head/neck primary tumour. Radical management with excisional biopsy or surgical resection of involved lymph nodes followed by external beam radiotherapy or concurrent chemoradiotherapy of involved neck and head/neck mucosa bilaterally results in long-term disease control in 50–60% of patients. The majority of these patients are cured, though field carcinogenesis places them at an increased risk of second tumours. The relative importance of surgical extirpation of neck nodes, bilateral irradiation of all head/neck mucosal sites up to the nasopharynx and the impact of chemotherapy administered before, during or after radiotherapy is still unclear in SQCCUP. Still, extrapolating data from patients with head neck cancer, we consider it appropriate for fit patients to be managed with aggressive multimodal therapy in order to maximise their chances of long-term disease control. Despite improvements in survival of CUP

patients managed with modern palliative chemotherapy and supportive care (Table 6), insights into the molecular biology of CUP are needed in order to identify the cellular signalling pathways responsible for primary tumour dormancy and early metastatic spread. CUP tumours, even when molecularly classified to primary tumour sites, may still harbour distinct genetic traits compared to metastatic tumours of known primary with typical biologic behaviour. These are potential targets for therapeutic modulation by rationally designed smart drugs that may ultimately result in improvement of patient outcome.

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