Granulocyte Colony-stimulating Factor Producing Oropharyngeal Squamous Cell Carcinoma

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Abstract. *Case Report: We report on the case of a 47-year old woman with granulocyte colony-stimulating factor (G-CSF)-producing relapsed oropharyngeal squamous cell cancer. Palliative immunotherapy with nivolumab was started. Absolute neutrophilic count increased during the course of immunotherapy and correlated with tumour progression. Under chemotherapy with weekly paclitaxel, dramatic tumour regression and decreasing absolute neutrophilic count were noted. G-CSF concentration in serum increased from 4.77 to 9.61 pg/ml during the final phase of tumour progression. Immunohistochemical staining of the initial biopsies showed that some of the tumour cells as well as infiltrating cells stained positively for G-CSF, and some of the tumour cells even stained positively for the G-CSF receptor. Conclusion: Leukaemoid reaction in malignant disease with increased neutrophilic granulocytes has been shown to correlate with dismal prognosis in other tumours. The role of G-CSF in progression and prognosis of head and neck squamous cell carcinomas is still unclear but in patients with these tumours there seems also to be a correlation between elevated G-CSF and poor prognosis. Further systematic evaluation of G-CSF secretion in this tumour entity should clarify the role and potential treatment possibilities for these tumours.*

There are many reports on granulocyte-colony stimulating factor (G-CSF)-producing tumours in the literature but only a few reported cases of this entity in head and neck carcinoma. Here we describe the case of a patient with a G-CSF-producing oropharyngeal tumour, discussing the outcome and poor prognosis associated with this tumour entity.

Case Report

We report on the case of a 47-year old woman with granulocyte colony-stimulating factor (G-CSF)-producing relapsed oropharyngeal squamous cell cancer after radical chemoradiation.

The patient presented to her general practitioner because of dysphagia and enlarged right cervical lymph nodes. Biopsy of an oral lichenoid appearing lesion was performed. Histology revealed a human papilloma virus-negative squamous cell carcinoma. Magnetic resonance imaging (MRI) showed a lesion in the right soft palate with extension to the right retromolar trigonum and suspected maxillar infiltration. Positron emission tomography-computed tomography confirmed bone infiltration of the alveolar process and the maxillary sinus. Field mapping biopsies were performed and confirmed infiltration of the uvula, right soft palate, and the right retromolar trigonum. The TNM classification (1) was cT4bcN2ccM0, tumour grade 2. Radical chemoradiation was performed for three cycles of platinum chemotherapy combined with radiotherapy for a total dose of 70 Gy in 35 fractions. However, positron-emission tomography-computed tomography revealed a metabolically active mass with central necrosis in the place of the previous tumour, thus confirming relapse. MRI confirmed a persisting tumour lesion of 3.5 cm × 2 cm in the right retromolar trigonum (Figure 1A). Histology upon re-biopsy confirmed relapse of disease.

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Figure 1. Magnetic resonance imaging of a 47-year-old woman diagnosed with relapsed oropharyngeal squamous cell cancer. A 3.5×2-cm lesion in the right retromolar trigonum (white dotted circle) can be seen (A), which increased in size during immunotherapy with nivolumab for seven cycles (*i.e.* 4 months) (B) and then diminished in size within 2 weeks (*i.e.* 3 weekly administrations of paclitaxel along with 8 mg dexamethasone) (C).

The tumour was deemed not operable and palliative immunotherapy with nivolumab at 3 mg per kg body weight was started. The absolute neutrophilic count (ANC) was $4.8 \times 10^6/l$ at that time. However, after initial subjective relief of symptoms, immunotherapy was stopped because of tumour growth after seven cycles (Figure 1B). An increase of ANC of $18.02 \times 10^6/l$ was noted, consisting exclusively of mature neutrophilic granulocytes without evidence of infection (Figure 2). Third-line chemotherapy with weekly paclitaxel at 90 mg/m^2 and 8 mg dexamethasone as premedication was started. After 2 weeks, dramatic tumour regression was noted clinically and upon MRI (Figure 1C), associated with decreasing ANC (Figure 2).

However, paclitaxel had to be stopped due to side-effects (*i.e.* worsening condition, anorexia) and the white blood cell count (WBC) was noted to be increasing.

A trial with nivolumab as rechallenge was performed. The dramatic response of the tumour to paclitaxel and dexamethasone within only 2 weeks left a faint hope that in fact pseudo-progression (*i.e.* infiltrating cells of the immune system) might have been the reason for increasing tumour size rather than tumour progression. A distinction between the two is not possible to make radiologically. After starting nivolumab again, the ANC decreased but the G-CSF concentration in serum increased from 4.77 to 9.61 pg/ml. The patient's condition deteriorated and treatment had to be stopped entirely. The patient died unfortunately, 1.5 years after primary diagnosis.

Immunohistochemical staining of the initial biopsies showed that some of the tumour cells as well as the infiltrating cells stained positively for G-CSF (Figure 3B), and some of the tumour cells even stained positively for the G-CSF receptor (G-CSFR) (Figure 3C).

Discussion

Leukaemoid reaction in malignant disease with increased neutrophilic granulocytes in the absence of leukaemia and infection is a well-known phenomenon (2). It has been shown that this may be due to secretion of G-CSF by the tumour, known as paraneoplastic syndrome, and that the prognosis of patients with tumour with increased neutrophilic granulocytes is dismal (3-5). Under physiological conditions, macrophages and monocytes are the cells which secrete G-CSF but it has been shown that tumour cells are capable of adopting this role (6). While ectopic production of G-CSF has been demonstrated for a vast variety of tumour types, only data of eight case reports are available on paraneoplastic G-CSF secretion in head and neck squamous cell carcinoma (7-14) (Table I).

In these published cases, and including our case, female patients were slightly over-represented (56%) compared with men (44%). The mean age was 59 years at the time of diagnosis. Outcome was poor, with patients dying on average 10 months after diagnosis (1.5 to 18 months).

The average WBC at the time of diagnosis in these patients was $35.8 \times 10^6/l$, which is roughly 4-fold the upper limit of normal. G-CSF was increased in seven cases, with an average of 197 pg/ml.

Histologically, all tumours were shown to be squamous cell carcinomas. In most studies, G-CSF was immunohistochemically confined to tumour cells. However, one study reported a negative result for G-CSF immunohistochemical staining (9) and in our study, as well as some of the tumour cells, some of the infiltrating leukocytes stained positively for G-CSF. There has been speculation that the rapid secretion of G-CSF from tumour cells may lead to negative staining results (9). Indeed, other

Figure 2. Neutrophilic granulocyte count (absolute neutrophilic count, ANC) and serum granulocyte colony-stimulating factor (G-CSF) concentration during the course of the disease.

Figure 3. Histochemistry and immunohistochemistry of serial sections (20×). A: Squamous cell carcinoma was seen upon haematoxylin-eosin staining. B: Tumour cells and some of the infiltration leukocytes stained positively for granulocyte colony-stimulating factor. C: Some of the tumour cells stained positively for granulocyte colony-stimulating factor receptor.

tumours associated with increased serum G-CSF concentration showed negative immunohistochemical staining in tumour cells (15, 16). However, serum G-CSF and WBC increase in parallel with tumour growth was reported in the majority of all published cases.

Moreover, in our patient, we noticed that ANC seemed to correlate with tumour growth. Interestingly, immunohistochemical localization of G-CSF was not only confined to tumour cells but was also found in tumour-infiltrating cells. This is a finding which has already been described in

Table I. Reported cases of granulocyte colony-stimulating factor (G-CSF)-producing head and neck squamous cell carcinomas.

Ref	Age, years	Gender	Histology	WBC, $\times 10^6/l$	ANC, $\times 10^6/l$	G-CSF, pg/ml	Cellular localization of G-CSF	Treatment	Survival (months)
(13)	61	M	SCC of the tongue	96.2	N/A	204	Tumour cells	Subtotal glossectomy with reconstruction. No chemoradiation	12
(10)	57	M	SCC of the tongue	22.7	N/A	>50	Tumour cells	Neoadjuvant chemoradiation, then glossectomy, segmental mandibulectomy and modified neck dissection followed by adjuvant chemoradiation	4.5
(14)	50	F	SCC of lower gums	32	N/A	250	Tumour cells	Surgery, then chemoradiation. Left mandibular and supra-omohyoid resection upon relapse	13
(9)	78	M	SCC of the tongue	21.7	86.4%	117	Negative in tumour cells	Oral chemotherapy. Patient declined surgery, radio- and chemotherapy	12
(11)	81	F	SCC of the buccal mucosa	41.8	Increased	N/A	Tumour cells	Chemoradiation and partial resection of the cheek and radical neck dissection	6
(12)	58	F	SCC of the tongue	21.38	84%	663	Tumour cells	Partial glossectomy and bilateral neck dissection	1.5
(7)	57	F	SCC of hypopharynx	46.3	89%	111	Tumour cells	Radiation with 4 cycles of cisplatin chemotherapy	12
(8)	45	M	SCC (CUP)	16.2	N/A	173	Tumour cells	Palliative radiotherapy (not operable, no chemo due to renal failure)	N/A
Our case	47	F	SCC of the oropharynx	23.53	90.9%	9.61	Tumour cells and some infiltrating cells	Not operable. Chemoradiation, then immunotherapy and paclitaxel chemotherapy upon relapse	18

ANC: Absolute neutrophil count; CUP: cancer of unknown primary; F: female; G-CSF: granulocyte colony-stimulating factor; M: male; N/A: not available; SCC: squamous cell carcinoma; WBC: white blood cell count.

other types of tumours (16). Staining was positive for G-CSFR in some tumour cells. The expression of both G-CSF and G-CSFR by the same cells in G-CSF-producing tumours has only been described twice, in a case of hepatocellular carcinoma (17), and a case of anaplastic pancreatic carcinoma (16).

In order to secrete G-CSF, cells are believed to have appropriate stimulation by tumour necrosis factor; interleukins 1, 3, and 4; granulocyte macrophage-colony stimulating factor; and interferon- γ (18-24). For G-CSF-producing tumours, one could speculate as to whether some tumour cells might become sensitive to an appropriately stimulating factor eliciting G-CSF secretion, or might be capable of producing G-CSF without appropriate stimulation, and whether infiltrating macrophages might produce G-CSF either through stimulation by tumour cells or as a bystander effect.

The findings of our case, compared to the other cases reported, suggest that these tumours seem to exhibit autocrine stimulation by secreting G-CSF and expressing G-

CSFR, hence increasing tumour growth/progression and explaining the dismal prognosis of G-CSF-producing tumours. In fact, there is evidence that this mechanism has a role in colon adenocarcinoma (25) and small-cell lung cancer cell lines *in vitro* (26). However, other studies did not find evidence for tumour stimulation through G-CSF (27).

The role of G-CSF in progression and prognosis of head and neck squamous cell carcinomas is still unclear but in these tumour types, there also seems to be a correlation between elevated G-CSF and poor prognosis. Increased rates of recurrence and metastasis were found in patients whose primary head and neck squamous cell carcinomas secreted granulocyte-macrophage-CSF (28).

In summary, we describe a case of a 47-year old woman with relapsed oropharyngeal squamous cell carcinoma and with only a short-lived response to immuno- and chemotherapy. A G-CSF-stimulated increase in ANC correlated with the clinical course and might explain the aggressive behaviour of the tumour and our patient's dismal prognosis. Further systematic evaluation of G-CSF secretion

in this tumour entity should clarify the role and potential treatment possibilities for these tumours.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

G.S., M.Z., R.G., S.V. and Y.K. performed all literature searches, designed the table and figures, and wrote the article. T.T.R. and M.D. performed immuno/histochemical stainings, and produced their figures and explanations.

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