The deleterious nature of the invasive front and dysplasia at margin in the long-term outcome from surgical treatment of squamous cell carcinoma of the head and neck

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Abstract
Introduction
The characteristic of the cells at the leading edge of malignancy may have a deleterious prognostic significance.

Materials and methods
A 10-year retrospective analysis was performed, data from 282 patients were reviewed and a detailed pathological review was undertaken. The data was entered into a database, which was independently validated for accuracy. Individuals recorded were monitored for five years from the initial surgical insult.

Results
The tumour–nodes–metastasis classification was validated in the follow-up study as a prognostic indicator. Transfusion had an adverse effect on survival and chance of recurrence. Dysplasia at margin, clearance (mm) of resection (i.e. surgical margin) and the nature of the invasive tumour front at the margin all had adverse effects upon the likelihood of cancer recurrence.

Discussion
The nature of the invasive front should be considered in any prognostic discussion and also when planning surgery. Transfusion triggers should be revised to avoid transfusion of blood products (which have cancer growth promoting properties). The survival rates after recurrence of disease is poor, in part, due to the entrenched adaptive behaviours of the residual tumour cells which manifest several areas of resilience (i.e. radiotherapy, chemotherapy) and which may have already invaded local important structures (making them resilient to surgery).

To improve the overall survival rates, we must address the surgical margin from the onset before considering adjunctive treatments which alter local vasculature (blood and lymphatic) and tumour spread patterns. Intraoperative margin analysis is important to address regions of concern before wound healing becomes entrenched and the chance to take corrective action is missed.

Introduction
The characteristic of the cells at the leading edge of malignancy may have a deleterious prognostic significance. Unlike the centre of cancer which may have a relatively poor blood supply, resulting in an anoxic environment and low metabolic activity, the invasive front of a tumour is bathed in the host milieu of oxygen and nutrients from both normal vasculature and the co-opted blood supply, enabling it to grow actively and persistently. The invasive front of cancers is also the battle ground where the often losing host defences (extracellular matrix and immune response) fail to destroy or limit the disease’s progress. The extent of the host response may well be indicated by the very nature of this edge of malignancy. In a well-developed host response, the edge of the lesion may well be encapsulated and the tumour limited by a fibro-immune reaction, whilst in more aggressive cancers this host response may be inadequate and the host defences may have several breaches from where the tumour gains privileged access to the rest of the body. Another important consideration is to not be limited to two-dimensional thinking because of our habituation towards viewing simple histological images from sectional analysis. It must be realised that the tumour exists as a three-dimensional entity with potentially viable chains of cells along all its borders ready to invade1–3.

The three-dimensional nature of the invasive border of a cancer may be appreciated in the Byrne classification system. The discohesive edge may not represent individual cells, but the infield view of some connected cells out of the plane of the histological section. In contrast, a cohesive front may represent a strong immuno-fibrous host response and a poorer adaptive ability of the tumour to overcome this1–3.

The nature of the invasive edge of a cancer has very important surgical ramifications. The often uncertain edge of the tumour leads to a survival margin of uncertainty which results in more adjacent normal tissue being excised to encompass cancer clearance2–4. This should be appreciated by both basic scientists and practicing clinicians (surgeons5, radiation oncologists) because there is always a finite probability of residual tumour growth manifesting later as a ‘recurrence’, the clones of which may have a degree of developed radio resistance. Objectively the primary curative treatment of head and neck squamous cell carcinoma is complete

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surgical ablation with clear margins evident on multiple histological sections. Radiotherapy after surgery does little to confer a significant survival improvement when a margin is involved. It is better to get things right the first time by appreciating the complex three-dimensional nature of cancer spread and the current limitations of intraoperative surgical excision guidance, which is still at a macroscopic level, whilst the disease infiltrates on a microscopic level.

The aim of the retrospective analysis was to investigate the factors affecting patient survival in head and neck carcinoma.

Materials and methods
A 10-year retrospective analysis was undertaken. Identical ‘intent to treat’ protocols were used to treat these patients. The study protocol was approved by the committee of the ethics for human research. Inclusion criteria included patients diagnosed with squamous cell carcinoma of the aerodigestive tract, complete patient episode details (clinical, surgical, radiotherapy and oncology) and follow-up for five years or until death. While exclusion criteria were applied when having incomplete medical records or loss to follow-up. Using these criteria, 282 sets of medical records were identified. Data from these records were reviewed and a detailed pathological review was undertaken; the data was entered upon a database which was independently validated for accuracy. Individuals recorded were followed for five years from the initial surgical insult. The database was validated by another medical practitioner before anonymisation and encryption.

All patients were operated upon with the primary objective of achieving a macroscopic clearance of 0.5–1.0 cm. Post-operative radiotherapy was given according to our standard protocols, if applicable.

Statistical analysis
The database was statistically analysed by an independent UK statistician. Regular briefing and debriefing meetings were held to explain possible hypotheses and review how they should be tested. Furthermore, interim analysis was used to modify hypotheses to try and refine how the data was interrogated to provide meaningful results.

Results
Demographic details of the 282 patients presenting for surgical treatment with head and neck squamous cell carcinoma are illustrated in Table 1. The demographics of our cohort show the typical sex and age distribution which is as expected. The Byrne classification was used to stratify the nature of the infiltrating edge of the cancer spread and the current limitations of intraoperative surgical excision guidance, which is still at a macroscopic level, whilst the disease infiltrates on a microscopic level.

Table 1 Demographic details of the 282 patients presenting for surgical treatment with head and neck squamous cell carcinoma.* UK socio-economic class classified from 1 (high) to 5 (deprived)

<table>
<thead>
<tr>
<th>Detail</th>
<th>Index</th>
<th>Detail</th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>90 female:192 male</td>
<td>Clearance at the superficial margin 3.95 ± 2.17 mm</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>65.49 ± 15.24</td>
<td>Clearance at the deep margin 3.87 ± 2.02 mm</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>90</td>
<td>Dysplasia at margin 52</td>
<td></td>
</tr>
<tr>
<td>Previous surgery</td>
<td>168</td>
<td>Type of neck dissection 132 (unilateral); 44 (bilateral)</td>
<td></td>
</tr>
<tr>
<td>Personal cancer history</td>
<td>83</td>
<td>Hard tissue resection 117</td>
<td></td>
</tr>
<tr>
<td>Heavy smoker</td>
<td>254</td>
<td>Length of surgery (hours) 7.89 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>Ex-heavy smoker</td>
<td>159</td>
<td>Previous chemo-radiotherapy 37</td>
<td></td>
</tr>
<tr>
<td>Heavy alcohol intake</td>
<td>100%</td>
<td>Local recurrence 52</td>
<td></td>
</tr>
<tr>
<td>Betel quid intake</td>
<td>14</td>
<td>Distant metastases 22</td>
<td></td>
</tr>
<tr>
<td>Dieting</td>
<td>32 on diet</td>
<td>Synchronous tumours 6</td>
<td></td>
</tr>
<tr>
<td>Socio-economic class*</td>
<td>4.57 ± 1.64</td>
<td>Tracheotomy 108</td>
<td>117</td>
</tr>
<tr>
<td>Depth of tumour invasion</td>
<td>9.47 ± 6.45 mm</td>
<td>MRSA status at 6 (admission); 16 (discharge)</td>
<td></td>
</tr>
<tr>
<td>Extracapsular invasion of LN</td>
<td>5 (bilateral)</td>
<td>Blood product transfusion 61</td>
<td></td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>41</td>
<td>Days in high dependency 1.84 ± 1.57</td>
<td></td>
</tr>
<tr>
<td>Neural invasion</td>
<td>15</td>
<td>Follow-up (to date) 40.75 ± 13.13 months</td>
<td>116</td>
</tr>
<tr>
<td>Bone/cartilage invasion</td>
<td>52</td>
<td>3 year survival 0.91 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>Severe dysplasia in field</td>
<td>63</td>
<td>5 year survival 0.87 ± 0.33</td>
<td></td>
</tr>
<tr>
<td>Discohesive pattern of invasion</td>
<td>113</td>
<td>Survival from first diagnosis (to date) 872.7 ± 328.2 days</td>
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cancer which was informed by dysplasia at the margin and sub-mucosal extensions (Figures 1–4).

There was a slight increase in tonsillar and tongue base tumours in the younger cohort, presumably attributable to early human papilloma virus infection. The series appear weighted towards earlier and less advanced oral and oropharyngeal tumours; hence, the decent three and five year survival figures (Figures 5–8).

The tumour–nodes–metastasis (TNM) system was validated as the prognostic measure T stage (both clinical and pathological) showed significance (p < 0.01) Log rank (Mantel-Cox) test with hazard ratio of 0.43; N stage (both clinical and pathological) showed hazard ratio of 0.44 and N+ disease (hazard ratio, 0.36) also showed significance (p < 0.05) Gehan-Breslow-Wilcoxon test. The group stage was also significantly correlated to survival (p < 0.001).

Local cancer recurrence was associated with poor survival (p < 0.001) and was significantly (p < 0.001) associated with previous surgery, clinical TNM, methicillin-resistant Staphylococcus aureus (MRSA) (on admission or discharge), length of surgery, depth of tumour invasion and its clearance both superficial (mucosal) and deep (soft tissue) margins, the need for a tracheostomy, total days in hospital post-operatively, pathological TNM and death from cancer.

Local recurrence was significantly associated with previous cancer history (p < 0.01) and dysplasia at the margin of resection (p < 0.05; Gehan-Breslow-Wilcoxon test with a hazard ratio of 0.418). Recurrence was significantly [p < 0.005 Log rank (Mantel-Cox) test] associated with lesions which had a discohesive front or invasive front rather than this with a cohesive or non-invasive front.

More infiltrative cancers will have a more advanced clinical stage, will take longer to remove and have a higher frequency of positive deep margins in the soft tissues with the patient requiring a longer post-operative hospital stay and often supplementary modalities such as radiotherapy in an attempt to salvage the margin or chemotherapy to palliate the patient for untreatable recurrence. Recurrence is associated with poor prognosis and reduced overall survival. Semantically, the slowly growing residual disease which becomes manifest is often termed a recurrence by the no novitiate, whilst it really is really residual disease left in an irradiated field leaving little room for successful treatment. The significant association of dysplasia at the margin of surgical resection with recurrence may represent true recurrence or

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Original research study

Figure 5: The effect of transfusion of leukocyte depleted packed red blood cells upon survival (in months) in 282 patients with head and neck squamous cell carcinoma treated with surgery.

Figure 6: Recurrence proportions and mucosal clearance (superficial surgical margin) of 5 mm or more in 282 patients with head and neck squamous cell carcinoma treated with surgical resection. Unlike a survival curve, this analysis shows that there is less recurrence with a bigger surgical margin.

Discussion

The compete assessment of the surgical margins at the time of resection with objective confirmation of complete removal of both tumour and liable field is most likely to be associated with long-term survival. This is an ideal that may not be often achieved, but the adverse consequences of minimal margin failure are often concealed by the ‘two-edged’ effect of scar formation; this serves not only to devitalise potential tumour seeds (making some dormant for later activation) but also to potentially shelter cancerous seeds from the local immune response. Unfortunately, post-operative radiotherapy does little to truly salvage an affected margin because survival tends to be worse if the lesion was completely removed initially with a clear margin of resection in the first instance; however, these patients and their physicians have little choice. The effects of transfusion delivered facilitators of tumour growth have been previously discussed as have their effects upon isolated cancer cell growth.\textsuperscript{12–16}

It may be assumed that in lesions with a cohesive front, it may be easier to surgically encapsulate a lesion with a closer margin of safety\textsuperscript{4} without leaving residual tumour that will manifest as a ‘recurrence’ in the fullness of time. However, in tumours that have a discohesive front, this may not be so simple and a greater margin (of safety) may have to be excised to achieve surgical ablation. As stated previously, in these cases, cells may often be left captive within the fibrous scar and may become dormant due to the nature of wound healing; however, discohesive cancer cells may also escape this reaction and later flourish. In the head and neck, this has important surgical, functional and survival ramifications since tissue volume is often at a premium in these locations with very little room for error.

Furthermore, the implication is that in a tumour with a discohesive edge abutting a vital structure, there may be a high chance of recurrence because the said structure is not removed. Our decision making is modified by the fact that some normal tissue structure provides a temporary barrier to tumour expansion until the tumour cell progeny gain the iterative adaptive ability to secrete the appropriate extracellular matrix breakdown and motility factors.

It cannot be assumed that the edge of a tumour is uniform in its cohesive and discohesive nature since this appears more to be a function of...

Figure 7: Recurrence proportions and deep clearance (surgical margin) of 5 mm or more in 282 patients with head and neck squamous cell carcinoma treated with surgical resection. Unlike a survival curve, this analysis shows that there is less recurrence with a bigger surgical margin.

Figure 8: The effect of an invasive or discohesive front (Byrne 3/4) and non-invasive or cohesive front (Byrne 1/2) on the survival of patients having recurrence.

The future
Optically directed measurements (e.g. microendoscopically, elastic scattering spectrographically or Raman spectroscopically) of tissue factors, such as VEGF expression or oxygen levels within the tumour and its invasive front, would help show if hypoxia itself is a stimulus for tumour growth and an independent factor in radiotherapy resistance.

For more immediate information it may be possible to consider optical diagnostic techniques such as laser capture microdissection, which harvests viable cells from the main tumour, its invasive front and lymphatic metastases (including extra-capsular spread) for rapid micro array or proteomic analysis.

It would also be useful to grow tumour cell lines from bulk of tumour, invasive front and metastases to allow molecular biological comparison to assess the efficacy of chemical reagents during drug development.

Particular interesting features to determine would be the expression of factors such as matrix metalloproteinase 1–9 at invasive front,

Discohesion probably manifests at specific points of host protection breakdown.

We are fortunate that in the many cases of theoretical margin breach, recurrence may not manifest because of the entrapment of a ‘limited’ number of tumour cells in scar tissue in a hypoxic environment, which results in their dormancy or non-progressive until senescence or breach in the limiting factors. This may occur through local trauma or wound healing enriching the local vasculature and spilling growth promoters within the micro-environment that are utilised by the tumour.

The survival rates after recurrence of disease is poor, in part, due to the entrenched adaptive behaviours of the residual tumour cells which manifest several areas of resilience (e.g. radiotherapy, chemotherapy) and which may have already invaded local important structures, making them surgery resilient. To improve overall survival rates, we must address the surgical margin from the outset before adjunctive treatments which alter local vasculature (blood and lymphatic) and tumour spread patterns. Intraoperative margin analysis is important to address regions of concern before wound healing becomes entrenched and the chance take corrective action is missed.

Abbreviations list
MRSA, methicillin-resistant *Staphylococcus aureus*; TNM, tumour–nodes–metastasis.