Research article

Morphological and immunophenotypic analysis of basal-like carcinoma of the breast

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Breast carcinomas present a heterogeneous group of tumours varying greatly with regard to histopathological classification, prognosis and treatment options. Recent research has identified a subtype of breast carcinoma that is associated with a poor prognosis and is most commonly found to be grade 3; these tumours are termed basal-like tumours due to their differentiation towards basal cell types.

Sixty cases of grade 3 ductal carcinomas not otherwise specified (NOS) were stained using immunohistochemical techniques with a panel of antibodies.

All cases were subjected to immunohistochemical and morphological analyses, and tumours were classified as either basal-like or ductal NOS.

Statistical analysis accompanied by sensitivity and specificity values showed cytokeratin 5 and vimentin to be the most useful antibodies for diagnosing the basal-like tumour. Morphological analysis found that a high mitotic count and presence of necrosis could be associated with the basal-like phenotype.

Results of this study have facilitated the development of an algorithm to allow identification and diagnosis of this tumour type.

Key words: basal-like, breast, immunophenotype.

Introduction

This paper investigates the immunohistochemical and morphological characteristics of the basal-like carcinoma of the breast. Breast cancer is a common malignant neoplasm comprising a large heterogeneous group of cancers that vary greatly in histological type and clinical course. Cancer of the breast is one of the most common human neoplasms; in 2004, there were 36,939 new registrations of breast cancer in women and 272 in men in the UK (http://www.cancer-screening.nhs.uk/breastscreen/breastcancer.html).

Biology of breast cancer

The normal female breast is a gland, composed of ducts with complex branching structures which originate in lobules and end in ducts at the nipple. The lobules and ducts are lined by two cell types: an inner, luminal/ductal cell layer and a peripheral basal cell layer.1 The basal cells are confined by a basement membrane which separates the epithelial component from the specialized breast connective tissue and adipose tissue.

Neoplastic progression has been shown to be associated with increased DNA damage and increasing malignant potential. It is thought that neoplasia begins in undifferentiated, reserve cells of the terminal duct lobular unit (TDLU), and that tumours show variable characteristics of differentiation representing the normal phenotypes of cells found in this region.2

Malignant tumours within the ductolobular system that are confined by the basement membrane are called ‘in situ’ and those which extend beyond this are termed ‘invasive’.3 Invasive carcinomas are potentially lethal as they can extend to involve local areas and also infiltrate into blood vessels and lymphatics, thereby metastasizing to distant areas of the body.

Classification

The classification of breast cancer is fundamental to understanding and treatment of the disease, with the prognosis of the disease being directly related to stage, grade and...
subtype. The group in which a tumour is placed will denote the course of action to be taken and the possibility of a variety of treatments being effective. Although there are many different types of breast cancer, most tumours (75–80%) are categorized in a single histological category—invasive ductal carcinoma not otherwise specified (NOS). This category is a heterogeneous group of cancers that do not show significant characteristics allowing them to be placed in one of the specialized categories (e.g. lobular or tubular).

Tumour grade is independent of tumour subtype classification. Grading a tumour involves assessing three components of the tumour morphology: tubule formation, nuclear pleomorphism and mitotic count. The grade of a tumour gives prognostic information that can be vital when deciding on a treatment plan. When tumours of equivalent stage are compared, high-grade tumours (less differentiated, lacking tubule formation, marked nuclear pleomorphism and showing high rates mitoses) are linked with a worse prognosis when compared with low-grade tumours (well differentiated, showing widespread tubule formation and minimal nuclear pleomorphism and a lower mitotic count).

**Basal-like carcinoma**

Recent research has identified a group of breast carcinomas which display specific characteristics associated with a high grade, hormone receptor (Oestrogen (ER) and Progesterone (PR)) negativity and ultimately a poor prognosis. These basal-like carcinomas are malignant tumours which show differentiation towards basal cells. This is in contrast to the majority of breast carcinomas which show differentiation towards luminal/ductal cells. Basal-like carcinomas can be identified as a specific entity using gene expression clustering, yet at present, there are no definitive criteria to routinely identify and diagnose these tumours.

**Clinical implications**

Much of the prognostic information and treatment options are related to tumour subtype. Therefore, much research has investigated the significance of diagnosing the basal-like carcinoma in terms of prognostic value. Basal-like carcinomas have been found to be mainly grade 3,10–12 and therefore, it is not surprising that this tumour type is associated with a poor prognosis when compared with all breast carcinomas. However, these studies have shown that basal-like carcinomas show a poor clinical outcome when compared with other grade 3 breast tumours.

It has been suggested that epidermal growth factor receptor (EGFR) targeted treatment may be of use in the treatment of the basal-like breast carcinomas. This therefore displays the necessity of accurate diagnosis for this specific subtype of breast cancer type.

**Immunophenotype**

Immunohistochemistry has revolutionized the process of classification; linking particular antibodies with a tumour type further characterizes the subtype of invasive carcinoma as well as identifying new types.

Research into this area is extensive; many studies have used different panels of antibodies in an attempt to define an immunophenotype for the basal-like carcinoma. At present there is no definitive immunohistochemical panel to classify the basal-like breast tumours; however, there is a consensus that one of the criteria is Oestrogen receptor/Progesterone receptor negativity.7, 11, 14, 15

Livasy et al.14 used a panel of antibodies on 18 basal-like carcinomas (confirmed using DNA microarray technology). All the tumours used in this study were ER and HER2 negative. Positivity was demonstrated for a number of markers, the most successful for characterizing these tumours being: vimentin (17/18), Cytokeratin 8/18 (15/18), EGFR (13/18) and Cytokeratin 5/6 (11/18). This study found that positivity for myoepithelial markers (SMA, p63 and CD10) was infrequent and not substantial to characterize this tumour type. These immunohistochemical findings suggest that vimentin may be of use as part of a panel of antibodies to diagnose this tumour type.14

A research group11 also investigated the immunohistochemical phenotype of the basal-like tumour of the breast. They studied 168 invasive breast carcinomas with ER and HER2 expression; from this the tumours were classified into four subtypes: luminal A, luminal B, basal-like and basal-like HER2 over expressing tumours. Further to this, the tumour subgroups were assessed for patterns of expression of P-Cadherin, p63, cytokeratin 5 (CK5), BCL2 and Ki67. The basal-like group was defined in this study by tumours which displayed ER and HER2 negativity and positivity for at least one basal marker (P Cadherin and/or CK5 and/or p63). Tumours that proved to be negative for all antibodies tested were not included in any of the four groups and therefore not included within the study; this approach varies from other researchers7 whose study would include a tumour that is negative for all antibodies within the basal group. Inconsistencies such as this between studies should be taken into account when reviewing literature as it causes variability of results.

**Morphology**

For routine diagnostic purposes, it is vital to be aware of specific morphological features that occur in the basal-like group. These could be recognized from a haematoxylin and eosin (H&E) section to invoke suspicion of this diagnosis, which could then be confirmed by use of an immunohistochemical panel.
Researchers have assessed the morphological features including histological grade that were found to be prevalent within the basal-like tumour group. This showed that these tumours were predominantly grade 3 tumours displaying a high mitotic count, geographic tumour necrosis, pushing margin of invasion, stromal lymphocytic response, high rates of nuclear pleomorphism and a lack of tubule formation.5, 9, 14

The morphological features found in the basal-like phenotype are largely consistent within these studies. However, they are morphological features that in general point towards a high-grade carcinoma, and specific diagnostic criteria have not been documented.

Materials and methods

Ethics
Prior to commencing the practical work involved with this project, it was necessary to obtain ethical approval from local NHS research and ethics committee (LREC) and the local NHS trust management (through the Research and Development department).

Cases
Sixty cases reported as ductal NOS grade 3 breast carcinomas were analysed in this study. Appropriate cases were identified from the database of the Department of Cellular Pathology at the John Radcliffe Hospital, Oxford. Paraffin embedded blocks were then retrieved from the archive stores; these blocks were previously prepared through the routine diagnostic procedures in place within the Department of Cellular Pathology.

Antibodies
Six antibodies were used in this study: CK5, CK7, CK14, vimentin, maspin and P-Cadherin. These antibodies were supplied by Vision Biosystems. Prior to the study, the dilutions were optimized: CK5, CK7 and vimentin were used at 1:400, P-Cadherin 1:200, Maspin 1:100 and CK14 1:80.

Oestrogen receptor, Progesterone receptor and HER2 status information was obtained from the cellular pathology computer database.

Suppliers of reagents and equipment
All reagents used on the Bond Maxx staining machines were supplied by Vision Biosystems. All equipment and other reagents were supplied by the Department of Cellular Pathology, John Radcliffe Hospital.

Table 1. Morphological features assessed on H&E sections

<table>
<thead>
<tr>
<th>Morphological feature</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubule formation</td>
<td>Determined by % of tumour that forms tubules</td>
</tr>
<tr>
<td>Grade 1 = more than 75</td>
<td></td>
</tr>
<tr>
<td>Grade 2 = 10–75</td>
<td></td>
</tr>
<tr>
<td>Grade 3 = less than 10</td>
<td></td>
</tr>
<tr>
<td>Nuclear grade</td>
<td>Graded 1, 2 or 3</td>
</tr>
<tr>
<td>Grade 1 = Nuclei slightly enlarged, minor variation in size, shape and chromatin pattern</td>
<td></td>
</tr>
<tr>
<td>Grade 2 = Nuclei distinctly enlarged, visible nucleoli, may be distinctly variable in size and shape</td>
<td></td>
</tr>
<tr>
<td>Grade 3 = Markedly enlarged nuclei, nucleoli often prominent, generally marker variation in size and shape</td>
<td></td>
</tr>
<tr>
<td>Nucleoli</td>
<td>Present or absent</td>
</tr>
<tr>
<td>Mitotic count</td>
<td>Actual number per mm²</td>
</tr>
<tr>
<td>Necrosis</td>
<td>Present or absent</td>
</tr>
<tr>
<td>Tumour infiltrating</td>
<td>None, mild, moderate and marked</td>
</tr>
<tr>
<td>lymphocytes</td>
<td></td>
</tr>
<tr>
<td>Lymphovascular</td>
<td>Present or absent</td>
</tr>
<tr>
<td>invasion</td>
<td></td>
</tr>
</tbody>
</table>

Controls
Normal breast tissue was used as control tissue in this study (as each of the antibodies being tested will be expressed in particular areas of normal breast tissue), and this was included in each run. Separate negative control were not required as internal negative controls can be seen in breast tissue structures such as stromal cells and vessels.

Immunohistochemical staining
Sections were stained using the Vision Biosystem Bond max staining machine. This staining machine includes steps to dewax, pretreat and stain slides.

A chain polymer-conjugated system was used for the immunohistochemical staining method in this study. This uses a primary antibody that attaches to the tissue antigens. A polymer-conjugated chain with attached secondary antibodies and peroxidase is then applied and attaches to the primary antibody. DAB (Di amino benzadine) is then added and acts as a substrate for the peroxidase; this reaction creates a brown colouration that is not soluble in water. Endogenous peroxidase in tissue is blocked prior to the addition of antibodies to prevent non-specific reactions.

On completion of staining, the slides were evaluated using light microscopy. Morphological features were assessed using an H&E slide. This process analysed morphological
The groups. HER2 status was not compared in this way.

The antibody expression scoring system used for assessing tumour slides

<table>
<thead>
<tr>
<th>Staining</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion</td>
<td>0 = no staining</td>
</tr>
<tr>
<td></td>
<td>1 = &lt;1% cells staining</td>
</tr>
<tr>
<td></td>
<td>2 = 1–10% cells staining</td>
</tr>
<tr>
<td></td>
<td>3 = 11–33% cells staining</td>
</tr>
<tr>
<td></td>
<td>4 = 34–66% cells staining</td>
</tr>
<tr>
<td></td>
<td>5 = 67–100% cells staining</td>
</tr>
<tr>
<td>Intensity</td>
<td>0 = no staining</td>
</tr>
<tr>
<td></td>
<td>1 = weak staining</td>
</tr>
<tr>
<td></td>
<td>2 = moderate staining</td>
</tr>
<tr>
<td></td>
<td>3 = strong staining</td>
</tr>
</tbody>
</table>

Reproducibility of the scoring system

The scoring system was statistically analysed using a Wilcoxon signed ranks test and a McNemar test when appropriate and found to be highly reproducible.

Immunohistochemical results

Following classification of cases into basal-like or ductal NOS groups, statistical analysis was used to quantify any difference in the immunoprofile of the two groups. It was clear from the data that a difference in antibody expression would be found between the two groups, which can be seen in Fig. 2. The score for intensity and percentage of cells stained were then added together to give a score from 0 to 8 for each antibody on each case. This method of scoring is used diagnostically in the laboratory for analysis of ER and PR and therefore allows comparisons to be made in this study.

HER2 scoring

Her2 scoring was performed by immunohistochemistry according to UK accepted diagnosis criteria. Patients with scores of 0 and +1 were considered negative, 3+ positive. Equivocal cases (2) were referred for fluorescence in situ hybridization (FISH).

FISH analysis

FISH analysis uses a fluorescently labelled cDNA (complementary DNA) sequences to calculate a ratio between the HER2 gene and chromosome 17 (which codes for the HER2 gene). A ratio of more than 2.2 (≥2.2 copies of the gene to each chromosome 17) means that the HER2 gene is over expressed.

Statistical calculations

On completion of analysis, 10% of the cases were re-scored to assess reproducibility. These values were compared with the original scores using a Wilcoxon signed rank test and if applicable McNemar test.

The full data sets of antibody expression were assessed using Mann–Whitney U tests to compare the medians of the groups. HER2 status was not compared in this way due to complexities in the scoring system with the culmination of FISH results. Therefore, analysis of HER2 relies on the use of sensitivity and specificity.

Immunohistochemical results were further analysed using positive and negative predictive values along with sensitivity and specificity values.

Results

Staining quality was of a very high standard and allowed visualization and interpretation of morphology in conjunction with the immunophenotype. This was essential as the basallike markers stained non-neoplastic basal cells of normal structures and ducts involved by ductal carcinoma in situ. These structures were identified and excluded from the assessment of marker positivity for the various antibodies. Examples of case staining can be seen in Fig. 1.

Classifying cases

Following morphological and immunophenotypic assessment of the 60 cases that had previously been diagnosed as ductal NOS, each case was classified as being either a ductal NOS or basal-like tumour. All of the cases were scored using a formula designed to separate basal-like characteristics from luminal/ductal features (CK5 + CK14 + vimentin + maspin + P Cadherin-CK7-ER-PR). There was a clear cutoff of tumours that had the greatest expression of basal cytokeratins (CK5/CK14), and these were classified as basal-like (15/60 of the research cases).
been described in the literature and will be examined further in the discussion.

Statistical analysis of immunohistochemical results
Using a Mann–Whitney U test, expression of each antibody from each group was analysed in turn. The results are as follows: ER <0.0005, PR <0.0005, CK5 <0.0005, CK14 <0.0005, vimentin <0.0005, Maspin <0.0005, P cadherin 0.004 and CK7 0.06. The majority of the antibodies tested displayed a difference of high significance (as defined by $P < 0.05$). It can be seen that the only antibody expression not to reach this level is CK7 ($P = 0.06$) (Table 3).

Positive predictive values (PPV), negative predictive values (NPV), sensitivity and specificity were also calculated for each antibody (Table 4).

Morphological results
In the assessment of basal-like tumours, it was noted that these are poorly differentiated tumours with almost no tubule formation. There are two distinct growth patterns: a solid, sheet-like growth and a trabecular arrangement (Figs 4 and 5). The latter was associated with prominent extracellular, hyalinized collagen reminiscent of basement

![Figure 1.](image1.png) Research case number 12: displaying the basal-like tumour phenotype with CK5, CK14 and vimentin positivity. Bar 100 μm.

![Figure 2.](image2.png) A graph showing the difference in antibody expression between the Ductal NOS and basal-like carcinoma groups.

![Figure 3.](image3.png) Research case number 28 displaying checker board arrangement (CK5 positive cells adjacent to CK5 negative cells. Bar 100 μm.)
membrane material. Often both of these areas were present in one tumour.

Morphological assessment included analysing six features of tumour morphology from an H&E section. These were tubule formation, nuclear grade, the presence of nucleoli, the presence of necrosis and the degree of infiltrating lymphocytes. Tubule formation, nuclear grade, mitotic count, presence of nucleoli and infiltrating lymphocytes were fairly consistent across both tumour groups. However, the presence of necrosis (Fig. 6) and mitotic count showed differences between the two groups. Necrosis was more prevalent in the basal-like tumour group compared with the ductal NOS group (80% compared with 20%, respectively, of the tumours displaying areas of necrosis). A difference was also apparent between the mean mitotic counts of the two tumour groups—the Ductal NOS group mean was much lower than that of the basal-like group. However, the range of the mitotic counts overlapped considerably (ductal NOS range $= 2–42 \, \text{mm}^2$, basal-like range $4–80 \, \text{mm}^2$).

### Discussion

This study set out to assess the phenotypic characteristics of basal-like carcinomas within a group of grade 3 ductal NOS carcinomas. Six antibodies were employed (CK5, CK14, vimentin, CK7, Maspin and P-Cadherin) to assess the heterogeneous group of grade 3 breast carcinomas, and were evaluated along with the ER, PR and HER2 status (also determined by immunohistochemistry).

### Immunophenotype of basal-like tumours

Grade 3 ductal NOS tumours of the breast can be subdivided based on morphological and immunophenotypic features. The results from this study confirm that there is a basal-like subtype of grade 3 ductal carcinomas that is ER negative and expresses basal cytokeratins. A characteristic immunohistochemical staining pattern has been shown to depict the basal-like tumour. This study has highlighted the antibodies that

### Table 3. Results from the Mann-Whitney U test to assess difference in antibody expression between the two tumour groups

<table>
<thead>
<tr>
<th>Antibody</th>
<th>U value</th>
<th>Level of significance (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>76.5</td>
<td>$&lt;0.0005$</td>
</tr>
<tr>
<td>PR</td>
<td>98.5</td>
<td>$&lt;0.0005$</td>
</tr>
<tr>
<td>CK5</td>
<td>4</td>
<td>$&lt;0.0005$</td>
</tr>
<tr>
<td>CK14</td>
<td>49.5</td>
<td>$&lt;0.0005$</td>
</tr>
<tr>
<td>Vimentin</td>
<td>24.5</td>
<td>$&lt;0.0005$</td>
</tr>
<tr>
<td>P Cadherin</td>
<td>179</td>
<td>$&lt;0.004$</td>
</tr>
<tr>
<td>Maspin</td>
<td>52.5</td>
<td>$&lt;0.0005$</td>
</tr>
<tr>
<td>CK7</td>
<td>245</td>
<td>$&lt;0.06$</td>
</tr>
</tbody>
</table>

### Table 4. Table showing PPV, NPV, sensitivity and specificity values for each antibody

<table>
<thead>
<tr>
<th>Antibody</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK5</td>
<td>100</td>
<td>96</td>
<td>87</td>
<td>100</td>
</tr>
<tr>
<td>CK14</td>
<td>89</td>
<td>96</td>
<td>53</td>
<td>98</td>
</tr>
<tr>
<td>CK7</td>
<td>31</td>
<td>100</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>Vimentin</td>
<td>76</td>
<td>95</td>
<td>87</td>
<td>91</td>
</tr>
<tr>
<td>Maspin</td>
<td>52</td>
<td>97</td>
<td>96</td>
<td>71</td>
</tr>
<tr>
<td>P Cadherin</td>
<td>26</td>
<td>10</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>Oestrogen receptor</td>
<td>0</td>
<td>42</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td>0</td>
<td>52</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>HER2</td>
<td>13</td>
<td>71</td>
<td>13</td>
<td>71</td>
</tr>
</tbody>
</table>
are the best discriminators for making the distinction between grade 3 basal-like and ductal carcinomas NOS. Statistical analysis reinforced the difference in antibody expression between these two tumour groups, a high level of significance (P = 0.005) was reached for six of the seven antibodies analysed (including ER and PR). PPV, NPV, sensitivity and specificity analyses confirm the diagnostic advantages of each antibody.

This study has shown CK5, vimentin and CK14 to be the three antibodies most successful in distinguishing the basal-like tumour. Both CK5 and vimentin have high sensitivity and specificity; this study suggests that the combination of CK5 and vimentin, in conjunction with ER, can be used to diagnose this tumour phenotype. ER is a useful indicator antibody as it is routinely performed on all breast carcinoma cases and shows no positivity in the basal-like group of tumours. CK14 has a relatively low specificity and therefore considered, by this study, to be less discriminatory than CK5 and vimentin. The use of CK5 is now well documented and accepted as a useful basal-like tumour marker to the extent that one study has suggested that just one basal cytokeratin marker (such as CK5) is all that is needed to diagnose this tumour type. Vimentin is not so widely acknowledged as a diagnostic marker for this tumour type; however, studies have started to recognize it as such, a result which this current study supports. It has also been suggested that positive vimentin staining denotes a preference for the basal-like tumours to metastasize to visceral tissues in preference to bone.

Previous studies have stated that CK14 expression could be used alone as a diagnostic marker for basal-like carcinomas. However, this is not supported by this study as over 26% of our basal-like group did not express CK14, but other accepted basal markers (CK5 and vimentin) did. This discrepancy may be due, in part, to the degree of staining necessary to qualify an antibody stain as positive. For example, Fulford et al. suggested that any positive CK14 staining present in 1% of tumour cells qualifies a positive result and allows a tumour to be placed in the basal-like group. This is in contrast to a study which stated that to be classed as basal-like, a tumour must show CK5/14 positivity in at least 70% of neoplastic cells. An earlier study on basal-like tumours classified CK5/6 staining as positive ‘if any cytoplasmic (weak or strong) and/or membranous invasive carcinoma cell staining was observed’. It can be seen that when drawing comparisons between research articles, methods of scoring staining is very variable, it is difficult, therefore, to assume any similarities or differences between results without taking this inconsistency into account.

Close examination of the basal-like groups of tumours in the current study shows that all of these tumours had more than 11% of neoplastic cells being positive for either CK5 or vimentin (the two most specific markers). From the results of this study, it can, therefore, be inferred that basal-like phenotype requires positivity of a basal marker in at least 10% of tumour cells. The UK National Health Service Breast Screening Programme (NHSBSP) states that a breast tumour should display over 90% of special-type differentiation to be placed within a special group and over 50% of special-type differentiation to be classified as ‘mixed type’. As with much of the recent literature, the classification in this current study would not substantiate ‘special group’ diagnosis according to the NHSBSP. However, much research has used criteria similar to that of the current study to describe basal-like tumours and have found this to have prognostic relevance. The most useful definition of a tumour type should be related to clinical outcome; it therefore seems justified to use this comparatively subtle expression of differentiation to diagnose this tumour type.

Basololumnar tumours
A recent study has suggested an additional subtype of breast carcinoma termed basololumnar tumours. Laakso et al. categorizes this tumour type as displaying 5–69% of tumour cells positive for CK5/14, with the added note that many of these tumours display a heterogeneous (checkerboard type) pattern of CK5/14 positive cells next to CK5/14 negative cells. As mentioned in the results, two cases from the current study (cases 2 and 28) were noted as displaying this checkerboard appearance on staining with CK5 and CK14 (Fig. 3); however, they fulfilled the criteria set by the current study to be categorized as basal-like. Laakso et al. found that classifying this basololumnar group as a new entity has clinical significance as it shows association with a poor prognosis when compared with the purely basal-like group. This may indicate that for accuracy of diagnostic and prognostic information, diagnosis of the basal-like tumour would benefit from a quantitative analysis of cells positive for basal cytokeratins as opposed to a straightforward positive or negative result.
In normal breast tissue, there is a difference between basal and luminal/ductal epithelial cells. This is mirrored in neoplastic progression, which starts with proliferation of undifferentiated tumour cells that differentiate towards displaying luminal/ductal or basal characteristics. It is rather simplistic to think that this is an either/or phenomenon. This differentiation (in both normal and disease states) should be considered more of a spectrum with cells characteristically becoming basal or luminal/ductal cell types, but with some that do not become so terminally differentiated. Considering this, it is not surprising that basal-like tumours will display some heterogeneity for basal markers and even a luminal/ductal phenotype (as seen by CK7 expression). The antibody CK7 is classified as a luminal/ductal marker and, therefore, it would not be expected to be positive in basal-like tumours. However, the current study has seen CK7 expression across the cohort (100% of basal-like tumours and 73.3% of ductal tumours) with no statistical difference between the subtypes. This finding supports previous research that found another luminal/ductal marker, CK8/18 was uniformly expressed by the majority of grade 3 breast carcinomas. The tumours in the current study that display the basoluminal phenotype (as described by Laakso et al. as heterogeneous, checkerboard pattern staining with basal cytokeratins) show diffuse positive staining with CK7. However, within the positive CK7 staining subpopulations of cells with weaker staining can be seen. This probably correlates with the staining pattern seen with basal cytokeratins indicating two subpopulations within the same tumour, but an immunohistochemical dual staining method would be needed to confirm this.

**Morphological findings of the basal-like carcinoma**

This study investigated certain areas of morphology of breast tumours on H&E sections. Many of the findings are not specifically associated with the basal-like subtype, but are associated with all grade 3 breast carcinomas (e.g. lack of tubule formation and high nuclear grade). As this cohort consisted entirely of grade 3 carcinomas, little difference was found in these criteria between the basal-like and ductal NOS groups.

However, this study has identified two morphological appearances of note that may be of use when diagnosing basal-like carcinomas; these are mitotic count and the presence of necrosis. The results show a higher mean count of mitoses in the basal-like group (27.6 mitoses/mm²) than the ductal NOS group (15 mitoses/mm²); however, the ranges of mitotic counts in the two groups do overlap. Therefore, a high mitotic count should raise awareness of this diagnosis that needs to be further confirmed.

Studies into the morphological appearance of the basal-like group of breast tumours show higher prevalence of necrosis in basal-like carcinomas compared with ductal NOS tumours. Rakha et al. found that high-grade

**Triple negative**

Basal-like tumours have been referred to as being ‘triple negative’ referring to lack of expression of ER, PR and HER2. The current study has shown that 87% of the basal-like group and 7% of the ductal group are triple negative, showing that this alone should not be used to define that basal-like group. Diagnosis should not be made solely on the lack of antibody/gene expression, but should rely on positive expression of known associated markers (e.g. CK5, CK14 and vimentin)

As part of the triple negative criteria, the lack of overamplification of the HER2 gene has been linked with the basal-like phenotype. This study has found that 13% (2/15) of basal-like tumours and 29% (12/45) of ductal tumours over-expressed HER2. This study shows that not all basal-like tumours are HER2 negative, and, therefore, this characteristic alone should not be used as a discriminatory factor when classifying this tumour. This supports a study that found 17/72 (23%) basal-like tumours (as defined by gene expression) to be HER2 positive.

![Figure 7. Suggested algorithm for identification and diagnosis of basal-like carcinoma of the breast.](https://academic.oup.com/biohorizons/article-abstract/1/1/19/233385)
comedo type necrosis was one of the common characteristics of the basal-like group of tumours (basal-like group defined as expressing CK5/6 and/or CK14). In the current study, the presence of necrosis is very different in the two groups. Eighty percent of the basal-like group showed necrosis compared with only 20% of the ductal NOS group. Despite the small numbers of cases involved in this study, a trend has been shown that on microscopic assessment of a routine H&E section should raise suspicion of the diagnosis of a basal-like tumour.

**Conclusion**

This study has further characterized the basal-like subtype of breast carcinoma using immunohistochemistry and led to the development of a diagnostic algorithm for making this diagnosis (Fig. 7). Confidence in the results would be increased by conducting further studies using a larger cohort and focusing on the antibodies and morphological features that this study has found diagnostically useful. This will facilitate the transfer of this diagnosis from the domain of research into routine histopathology laboratories which will result in improved patient diagnosis, prognostication and, hopefully, outcome.

**Acknowledgements**

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**Resources**

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**References**