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ANATOMICAL PATHOLOGY

# Invasive lobular carcinoma of the breast: assessment of proliferative activity using automated Ki-67 immunostaining

Benjamin Dessauvagie[1,2,3], Anitha Thomas[1,3], Carla Thomas[1,2], Cleo Robinson[1,2], Marais Combrink[1,2], Vanitha Budhavaram[4], Bindu Kunjuraman[4], Katie Meehan[2], Greg Sterrett[1,2,3], Jennet Harvey[1,2,3]

*[1]Breast Subspecialty Group, Department of Anatomical Pathology, PathWest Laboratory Medicine WA, Perth, WA, Australia; [2]School of Biomedical Science, University of Western Australia, Crawley, WA, Australia; [3]Division of Pathology and Laboratory Medicine, UWA Medical School, University of Western Australia, Crawley, WA, Australia; [4]Department of Surgery, Sir Charles Gairdner Hospital, Nedlands, WA, Australia*

**Summary**

Invasive lobular carcinoma (ILC) is almost always classified as Nottingham histological grade 2. Despite this, prognosis is markedly varied, with some ILCs behaving more akin to grade 3 invasive ductal carcinoma (IDC). Methods to separate these aggressive ILCs are needed. Digital image analysis (DIA) of the Ki-67 biomarker has potential in this regard; thus, we sought to determine the feasibility of its use for automated evaluation of ILC. An initial pilot study demonstrated no ILC specific changes were required to our Ki-67 DIA algorithm for reproducible results. Subsequently, 42 consecutive cases of ILC were evaluated by visual mitosis counting in H&E stained sections and by DIA on Ki-67 stained sections. Ki-67 proliferative index (PI) DIA showed significant correlation with visual mitosis counting on H&E stained sections ($r_s$=0.63; $p$<0.05), significant strong correlation ($r_s$=0.78; $p$<0.05) and substantial agreement (κ=0.62) with manual/visual Ki-67 assessment and significant positive associations with grade, nodal status and 'pleomorphic' ILC subtype, and a wide stratification of values in classical/grade 2 ILC. In conclusion, DIA of Ki-67 PI in ILC is feasible, correlates with mitotic index, manual/visual Ki-67 PI and clinicopathological variables. The broad stratification of Ki-67 PI in classical/grade 2 ILC supports its practicability as a biomarker with prognostic and predictive potential, although large studies with outcome data are required for validation.

*Key words:* Breast neoplasms; computer assisted diagnosis; image processing; cell proliferation.

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## INTRODUCTION

Classical invasive lobular carcinoma (ILC), characterised by dyscohesive tumour cells with intermediate sized, uniform nuclei and low mitotic rate invading singly, in linear files or as aggregates in a fibrous stroma, accounts for 5–15% of all breast carcinomas.[1,2] In a small proportion of cases, nuclei display greater pleomorphism and high nuclear grade and such tumours are categorised as pleomorphic lobular carcinomas (pILC).[3] Nottingham histopathological grade, incorporating the assessment of tubules, nuclei and mitoses, is a widely used and validated prognostic tool for breast cancer prognostication and in predicting response to chemotherapy.[2–4] However, given their characteristic morphology, classical ILCs are almost always grade 2.[1,5] When compared with grade matched invasive duct carcinoma (IDC) of no special type, some cases of classical ILC have a propensity for worse long term outcome more akin to higher grade tumours.[5] Better methods of risk-stratification in ILC would be valuable since chemotherapy (and its associated risks) might be avoided in some cases with excellent prognosis, whereas tailored, more aggressive therapy could be administered in tumours likely to progress.[1,5,6]

Assessment of proliferation by various methods has demonstrable value in prognostication and prediction in breast carcinoma generally[4,7–9] and of the three components of the Nottingham histopathological grade, mitotic index (MI) has the strongest prognostic and predictive power.[2,4] Ki-67 immunohistochemistry, which identifies a cell proliferation-associated nuclear antigen, is in routine use in most laboratories and can be assessed efficiently and reproducibly using digital image analysis (DIA).[10] Numerous studies have demonstrated prognostic and predictive roles for Ki-67 in breast carcinomas with poorer outcomes and better responses to chemotherapy associated with higher Ki-67 levels.[7–9,11] Unlike MI, the Ki-67 proliferative index (PI) is reported as a percentage of all tumour cells and therefore adjusts for tumour cellularity. For this reason Ki-67 has been considered a superior index of proliferative activity.[12] However, clinical application of Ki-67 is hampered by a perceived lack of accuracy, reproducibility and standardisation and by lack of consensus in regard to pre-analytic and analytic factors including specimen type (core vs excision), antibody clone used (different clones result in different results), method of quantification (visual vs manual counting) and in interpretation (variable cut-off values separating 'high' and 'low' Ki-67 PI).[13] More recently, commercially available

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RNA expression profile molecular tests (such as Oncotype Dx, MammaPrint and PAM 50), which output strong and reproducible prognostic and predictive information, are being utilised to stratify breast carcinoma risk and tailor therapy.[14–16] However, these tests, which actually rely heavily on the status of proliferation related genes, are expensive and not widely available.[14–16]

Almost all breast carcinoma proliferation and molecular prognostication studies have been performed on cohorts comprised predominantly or exclusively of IDCs, with relatively few studies specifically addressing lobular carcinomas. The characteristic morphology of these tumours is a potential confounding factor in proliferation analysis, and more broadly, direct application of results from studies devoid of lobular cases to an ILC patient in the clinic requires a degree of caution.

Therefore, primarily as a technical project, we aimed to determine if our method for automated assessment of Ki-67 PI in breast carcinoma could be specifically optimised for ILC and to compare routine mitosis counting in lobular carcinoma cases with automated Ki-67 PI assessment as a quality check. A secondary aim was to correlate this proliferation data with standard clinicopathological prognostic indicators.

## MATERIALS AND METHODS

### Case selection

H&E stained sections and pathology reports of 42 consecutive patients with invasive lobular carcinoma over a one year period (2013) were retrieved from the archives of the Department of Anatomical Pathology, PathWest Laboratory Medicine WA, QEII Medical Centre. Clinical follow-up data was obtained by review of the corresponding Sir Charles Gairdner Hospital case notes. Approval for the project was obtained from the Sir Charles Gairdner Hospital Audit and Quality Improvement department (quality activity numbers 8053 and 21772).

### Immunostaining

A representative block was selected based on high tumour cell content. Ki-67 immunostaining of 4 µm sections was performed according to previously described methods.[10] Briefly, staining was conducted on an automated platform (Ventana Ultra; Ventana, USA), with heat induced antigen retrieval, incubation of Ki-67 antibody (MIB1, M7240, 1/400 dilution; Dako, Denmark) for 60 min, with DAB (Ventana) as chromogen, and visualised by the Ultraview DAB detection kit (Ventana). Positive and negative controls were included in each run.

### Reviewed visual counting of mitoses

Consensus visual H&E mitotic index evaluation was conducted by at least two pathologists at a multiheader microscope. Mitotic figures were counted using a 40× objective (0.55 mm diameter), in 10 non-overlapping fields, targeting areas of highest mitotic activity often at the advancing edge of the tumour. Mitotic figures were only counted if they had recognisable chromatids in prophase, metaphase, anaphase or telophase, lacked an intact nuclear membrane and were unanimously agreed by all pathologists to represent mitoses.

### Manual/visual Ki-67 evaluation

Ki-67 slides were scanned using Aperio ScanScope AT (Leica Biosystems, Germany). To conduct the manual/visual Ki-67 evaluation, each case was viewed onscreen in Aperio ImageScope software (Leica Biosystems) and screen-shots from the area of highest proliferation (the so-called 'hotspot') were taken by a pathologist at 20× magnification. These screen-shots were printed in colour and then visually assessed by a pathologist, manually scoring positive tumour cells as a percentage of all tumour cells across at least 500 cells, according to international recommendations.[13]

### Ki-67 digital image analysis

Ki-67 DIA was performed as previously described.[10] Briefly, the 'hot-spot' areas on the scanned slides were outlined on screen by a pathologist for each case using the selection tool within Aperio ImageScope software aiming to exclude benign elements and areas of abundant stroma from the analysis. The selected area was assessed using the Aperio IHC nuclear algorithm. The algorithm is designed to exclude populations of small cells (such as lymphocytes) and spindled cells from the count and identify tumour nuclei, classifying them as negative (0) or weak (1+), moderate (2+), or strongly stained (3+). The algorithm provides a four-colour mark-up image for visualisation of positive staining, which can be viewed on screen. For estimation of Ki-67 proliferative index (PI) any level of Ki-67 staining recognised by the Aperio Algorithm was considered positive and reported as a percentage of all tumour nuclei. At least 1000 cells were counted in each analysis (Fig. 1), as per our own quality assurance findings.[17]

Given the characteristic morphology of ILC, we considered the possibility that some tumour cells maybe incorrectly identified as stromal or lymphoid cells or vice versa. Therefore, the default IHC nuclear algorithm parameters were modified to further exclude elongated nuclei (i.e., stromal cells) and the threshold for nuclear size was increased to improve exclusion of small nuclei (i.e., lymphocytes). All other algorithm parameters were kept at the default settings. The modified algorithm resulted in a visible improvement in the on-screen mark-up of tumour, stromal and lymphoid cells; however, a complete exclusion of stromal nuclei and lymphocytes was not possible without compromising the identification of tumour nuclei. In addition, statistical evaluation of the modified versus default algorithms showed a very strong and significant correlation in the Ki-67 PI (Spearman coefficient of 0.98; $p<0.001$) with a line of correlation of roughly 1:1 (Fig. 2). Thus the original (default) Aperio algorithm settings were used for the analyses in this study.

### Statistical analysis

Spearman rank coefficient was applied to analyse the correlation between proliferation methods. The slope (m) of the line of correlation was determined for each comparison (y=mx) to give an indication of the ratio between the y- and x-axis values. Kappa statistics were applied between the visual and digital evaluations of Ki-67 as a measure of accuracy.

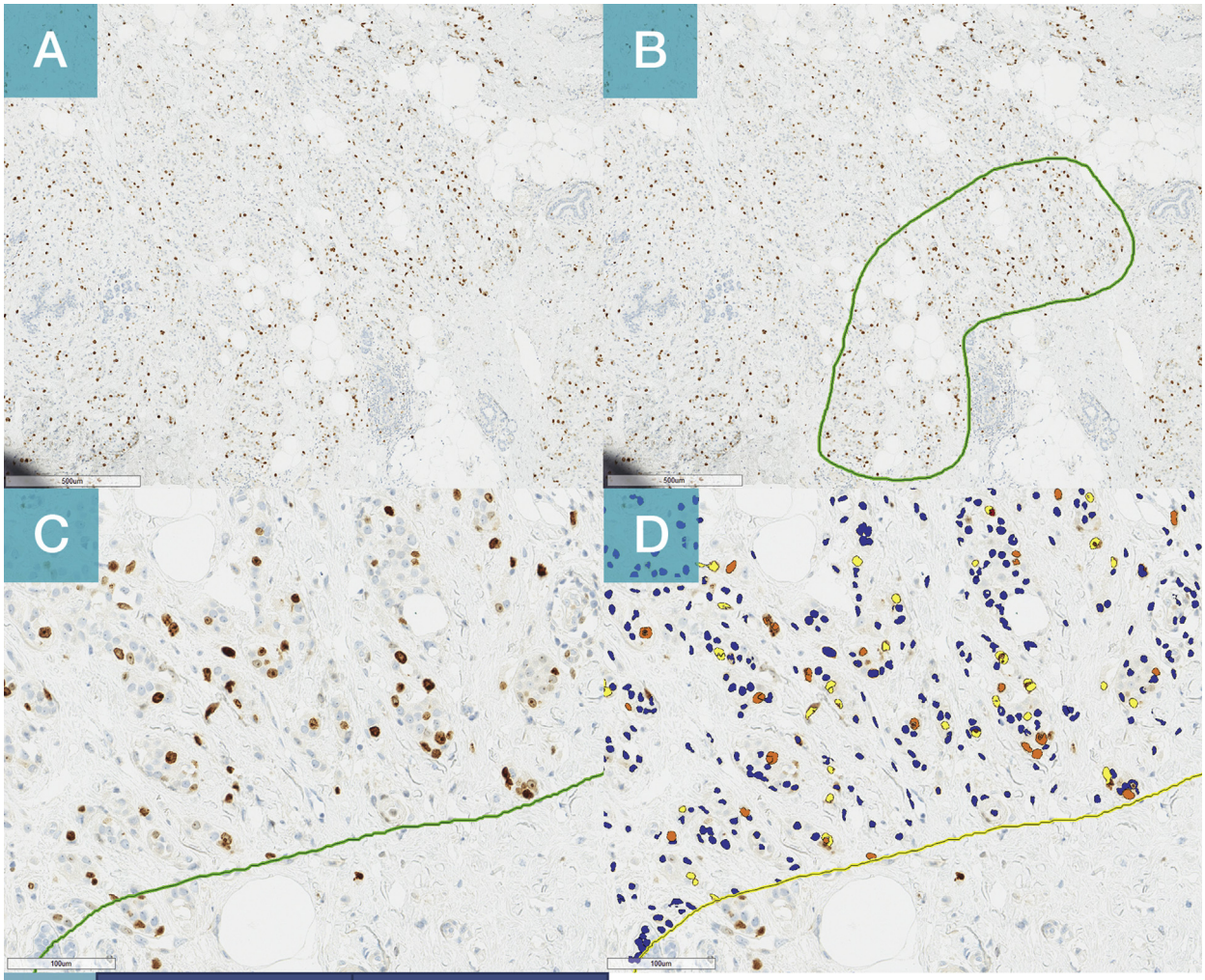
For analysis against clincopathological variables, the cohort was subdivided by the variables of Nottingham histological grade, oestrogen receptor (ER) status, progesterone receptor (PR) status, HER2 status, classical or pleomorphic type, and nodal status. Ki-67 PI mean and confidence interval were calculated for each category. Means were compared using t-tests for two variables or one-way analysis of variance (Classic or Welch's if unequal variance) for three or more variables.

A $p$ value of <0.05 was considered significant for all analyses.

## RESULTS

### Cohort demographics

The cohort consisted of 42 females aged between 38 and 78 years with a mean age of 60 (Supplementary Table 1, Appendix A). Three tumours were Nottingham grade 1, 35 were Nottingham grade 2 and four were Nottingham grade 3. Thirty-seven tumours were classified as classical ILC while five cases met criteria for pILC. To be considered 'pleomorphic' the tumour cells displayed nuclei at least two to three times the size of a lymphocyte or benign ductal cell nucleus, showed marked variation in nuclear size and shape, with clumped or vesicular nuclear chromatin and the presence of nucleoli (thus meeting criteria for a nuclear pleomorphism score of 3 in the Nottingham grading system). Four of the pILC cases were classified as Nottingham grade 3 and one was Nottingham grade 2. Tumour size for all cases ranged from 2 mm to 150 mm. All tumours were ER positive and all but one grade 3 pILC were HER2 negative. Where axillary node evaluation was performed, at least one metastasis was




E

| Attribute | Value |
|---|---|
| Percent | 20.802 |
| Intensity | 2 |
| 3+% | 1.50376 |
| 2+% | 9.94152 |
| 1+% | 9.35673 |
| 0% | 79.198 |
| Intensity Value | 183.261 |
| # Cells | 1197 |

**Fig. 1** Ki-67 digital image analysis. (A) Aperio ImageScope Ki-67 immunostained section of invasive lobular carcinoma (ILC) (5×). (B) The 'hot-spot' area was outlined onscreen by a pathologist using the selection tool within Aperio ImageScope software (5×). (C) Magnified image (×20). (D) Markup image generated using the Aperio IHC nuclear algorithm within the selected 'hot-spot' area. (E) Percentage of positive stained nuclei displayed in table format within Aperio ImageScope software.

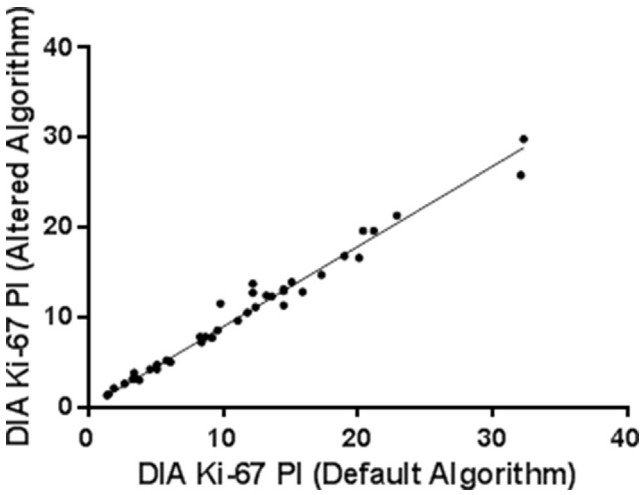



**Fig. 2** Correlation between automated DIA Ki-67 PI using the original (default) algorithm settings and the modified algorithm settings showed a significant, very strong Spearman correlation of 0.98 ($p$<0.001).

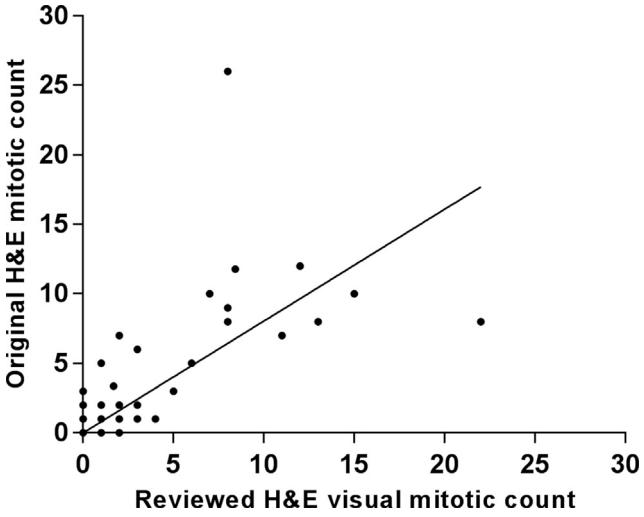



**Fig. 3** Comparison of the reviewed visual mitosis count with the originally reported mitosis count on H&E stained sections showed a significant, very strong Spearman correlation of 0.80 ($p$<0.0001). The slope of the line of correlation was 1.25 indicating, on average, 25% more mitoses identified on the reviewed count.

identified in 16 of 38 cases. Metastases were reported in all cases of pILC.

### Comparison of reviewed mitotic count and originally reported mitotic count

The originally reported mitotic counts and the reviewed mitotic count in H&E sections performed during this study showed very strong and significant correlation, with a Spearman coefficient of 0.80 ($p$<0.0001) and a line of correlation of 1.25 indicating, on average, 25% more mitoses identified on the repeat count (Fig. 3).

### Comparison of manual/visual Ki-67 and DIA Ki-67 results

The manual/visual Ki-67 PI results showed a significant, strong correlation with DIA Ki-67 PI with a Spearman coefficient of 0.78 ($p$<0.0001) (Fig. 4). The slope of the line of correlation was 0.7, indicating DIA Ki-67 PI results were, on

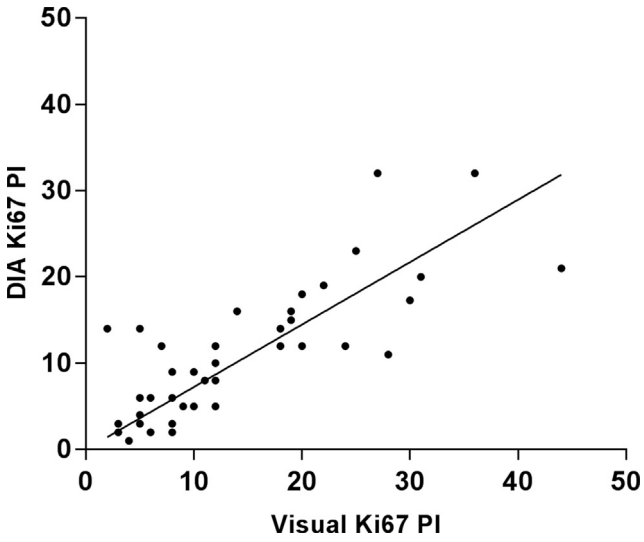



**Fig. 4** Comparison of visual/manual Ki-67 and DIA Ki-67 results showed a significant, strong Spearman correlation of 0.78 ($p$<0.0001). The slope of the line of correlation was 0.7, indicating DIA results were, on average, slightly lower at 70% of the visual/manual scores.

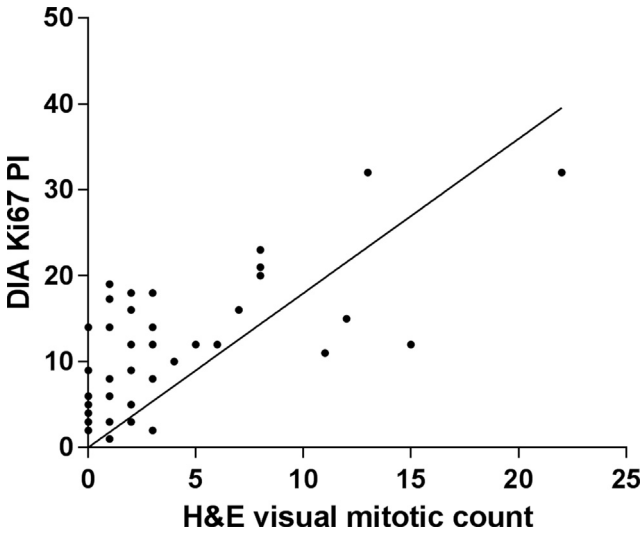



**Fig. 5** Reviewed visual mitotic count on H&E sections compared with DIA on Ki-67 proliferation index showed a significant, strong Spearman correlation of 0.63 ($p$<0.001).

average, slightly lower at 70% of the manual/visual scores. Substantial agreement between manual/visual and DIA Ki-67 PI ($\kappa$=0.62), was demonstrated when data were dichotomised at <10% versus ≥10% (a clinically relevant threshold identified by Aleskandarany *et al.*).[9]

### Comparison of reviewed mitotic counts with DIA Ki-67 results

The reviewed mitotic count in H&E sections showed a strong and significant correlation with DIA Ki-67 PI with a Spearman coefficient 0.63 ($p$<0.001) (Fig. 5).

### Overview of proliferation measures in Nottingham grade 2 classical ILC

There were 34 grade 2 classical ILCs in our cohort. The range of reviewed visual mitotic counts was 0–15 per 10 high power fields (HPFs). The median and mean number of

**Table 1** Ki-67 proliferative index and clinicopathological parameters

| | Mean Ki-67 (95%CI) | *p* value |
|---|---|---|
| Grade | | <0.001 |
| 1 | 5.7 (−3.1–14.4) | |
| 2 | 9.8 (7.9–11.8) | |
| 3 | 27.0 (17.7–36.3) | |
| ER status | | N/A |
| Positive | 11.2 (8.8–13.5) | |
| Negative | All cases positive | |
| PR status | | >0.05 |
| Positive | 11.1 (8.8–13.4) | |
| Negative | 11.8 (−10.0–33.6) | |
| HER2 status | | N/A |
| Amplified | Only 1 case amplified | |
| Non-amplified | 11.0 (8.6–13.4) | |
| Type | | <0.0001 |
| Classical | 9.2 (7.4–11.0) | |
| Pleomorphic | 25.6 (18.2–33.0) | |
| Nodal status | | 0.002 |
| Positive | 16.3 (11.7–20.8) | |
| Negative | 7.8 (5.7–9.9) | |

CI, confidence interval; ER, oestrogen receptor; N/A, not applicable; PR, progesterone receptor.

mitoses per 10 HPFs was 2 and 3, respectively. Thirty-one tumours (91%) had a score of 8 or fewer mitoses per 10 HPFs (the cut-off for a mitotic score of 1 in the Nottingham index on a 0.55 mm field diameter microscope) with three cases in the range for a mitotic score of 2.

The observed range for DIA Ki-67 PI was 0–20% with a median PI of 9% and a mean of 10%. Seventeen cases (50%) had a Ki-67 PI of <10% and 17 cases (50%) had a Ki-67 PI of ≥10% (a clinically relevant threshold identified by Aleskandarany *et al.*).[9] Eight cases (24%) had a Ki-67 PI of ≤4% and 26 cases (76%) had a Ki-67 PI of >4% (a clinically relevant threshold identified by Carbognin *et al.*).[18]

### Overview of proliferation measures in pleomorphic ILC

The mean visual mitosis count on H&E sections in the pILC cases was 12 per 10 HPFs with a range of 8–22 per 10 HPFs. Three cases were classified as mitotic score 1, one case as mitotic score 2 and one as mitotic score 3. All pILC cases had a Ki-67 ≥10%. The mean DIA Ki-67 PI of the pILC cases was 26% with a range of 21–32%.

### DIA Ki-67 PI and correlation with clinicopathological parameters

Ki-67 PI in ILC showed significant positive correlations with tumour grade, pleomorphic subtype, and nodal involvement (Table 1). There was no significant difference in the mean Ki-67 PI of PR positive/negative cases. As all cases were ER positive and only one case was HER2 positive, no meaningful analysis in regard to these variables was possible.

Follow-up data was obtained for 35 of the 42 cases. The length of follow-up ranged from 13 to 60 months following the initial surgery with a median length of follow-up of 48 months and an interquartile range of 12 months. No local recurrences were observed. Distant metastases were reported in four cases at between 22 and 41 months (Table 2). One of these metastatic cases was a Nottingham grade 3 pILC, all others were classical type, Nottingham grade 2 tumours. Tumour sizes in the metastatic cases ranged from 55 mm to 120 mm, all were ER/PR positive, HER2 negative, all had positive axillary nodes at the time of diagnosis and all were treated by mastectomy with clear margins. Two women subsequently received chemohormonal therapy, one received hormonal therapy alone, and no information was available for one woman. All four metastatic cases had a DIA Ki-67 PI of ≥10% (Fig. 6).

## DISCUSSION

ILC is an important breast cancer subtype, representing approximately 10% of all invasive breast cancers. Given their usual morphology, ILCs are typically Nottingham grade 2

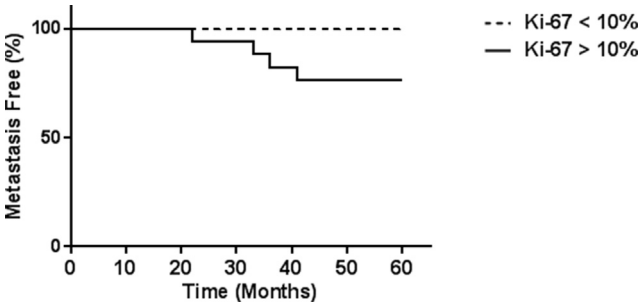



**Fig. 6** Kaplan–Meier graph demonstrating metastatic events across all cases dichotomised around a DIA Ki-67 PI of 10%. All events occurred in cases with an elevated Ki-67.

**Table 2** Cases with local recurrence or distant metastases on follow-up

| | Case number | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 20 |
| Tumour type | Classical ILC | Classical ILC | Pleomorphic ILC | Classical ILC |
| Tumour grade | 2 | 2 | 3 | 2 |
| Tumour size, mm | 55 | 75 | 120 (multifocal) | 60 |
| Receptor status | ER+/PR+/HER2− | ER+/PR+/HER2− | ER+/PR+/HER2− | ER+/PR+/HER2− |
| Lymph node status | 3/8 | 20/23 | 13/19 | 2/11 |
| Original mitotic index (per 10 HPFs) | 7 | 2 | 9 | 1 |
| Repeat visual mitosis index (per 10 HPFs) | 11 | 3 | 8 | 1 |
| Manual/visual Ki-67 PI, % | 28 | 7 | 25 | 22 |
| DIA Ki-67 PI, % | 11 | 12 | 23 | 19 |
| Length of time of follow up, months | 41 | 36 | 24 | 36 |
| Length of time to local recurrence, months | N/A | N/A | N/A | N/A |
| Length of time to metastasis, months | 41 | 33 | 22 | 36 |
| Site of distant metastasis | Liver | Peritoneum | Brain, bone, liver, iliac nodes | Bone |

and have generally favourable clinicopathological features, yet a proportion have a poor long term outcome.[5] Therefore, standard clinicopathological variables fail to adequately stratify risk of these tumours necessitating additional methods for prognostication.[1,5,6] This study investigated the technical feasibility of the use of automated Ki-67 PI, a proliferative marker with strong prognostic power in invasive breast carcinoma generally, specifically in ILC.[8,9,11,12,19]

We found that the mitotic count recorded in the original report strongly correlated with the reviewed consensus mitotic count. While this is a reassuring quality assurance finding, indicative of a strong reproducibility of mitotic evaluation within our department, a confounding factor is the propensity for classical ILCs to intrinsically display a low mitotic count within a narrow range. This narrow spectrum is the inherent limitation of MI (and therefore grade) as a predictor of outcome in the great majority of ILCs.

We have demonstrated that automated DIA of KI-67 PI in ILC is technically feasible utilising an 'off-the shelf' (default) algorithm at factory pre-set parameters with results demonstrating the expected positive associations with mitotic index, manual/visual Ki-67 evaluation, Nottingham grade, pleomorphic subtype and nodal status.

Digital evaluation of Ki-67 PI offers a number of advantages over conventional manual/visual methods of Ki-67 assessment in tissue sections, in particular its speed and efficiency of evaluation,[10,20] ease of incorporation into laboratory work-flow,[10] and excellent intraobserver, interobserver and interlaboratory agreement and capacity for quality assurance.[17,21] We note that our digital Ki-67 PI assessments were, on average, lower than Ki-67 values obtained by manual/visual counting. We believe this is multifactorial, due in part to the lower numbers of cells evaluated manually (500–1000 cells versus 1000 or more; we have demonstrated that a progressively increasing the number of cells for Ki-67 PI evaluation results in a progressive decrease in the Ki-67 PI of a particular case)[17] and that DIA can incorrectly label negatively stained lymphocytes and stromal cells as tumour cells, artificially lowering the tumour Ki-67 PI. To reduce this risk, we limit our digital Ki-67 evaluation to less than 16,000 cells and purposely avoid selection of stroma and lymphocyte rich areas when conducting digital Ki-67 PI evaluation.[10,17,21]

We demonstrated that DIA Ki-67 PI provides a broad spectrum of results, even in low mitotic ILC, which has the potential to offer better risk stratification and thus guide therapeutic options. For example, Ki-67 at a cut-off of $\geq$10% was able to divide our grade 2 classical ILCs into two equal sized groups (50% vs 50%) in a similar vein to the 50:50 split observed by Aleskandarany *et al.*[9] These investigators showed that a <10% Ki-67 PI in grade 2 invasive breast cancers (of all histological subtypes) identified a group of tumours with biological behaviour akin to grade 1 tumours for which patients could potentially be spared aggressive therapy and its associated morbidity. In another example, roughly 24% of our grade 2 classical ILCs had a Ki-67 PI of $\leq$4%, a prognostically significant Ki-67 PI cut-off observed by Carbognin *et al.* in an ILC specific cohort.[18] In their study, low proliferation ILC patients had significantly improved 10 year overall and disease free survival compared to ILCs with >4% Ki-67 PI.

Our study is primarily a technical feasibility and quality assurance study and is thus limited by small numbers of cases with insufficient outcome data for reliable clinical interpretation. Specifically, there were only four significant events documented in 60 months of follow-up of our study cohort. It is interesting to note, however, that all four cases with distant metastases at follow-up had a Ki-67 PI that is considered 'high' by several groups, including Aleskandarany *et al.* and Carbognin *et al.*[6,9,18]

There are very few studies specifically addressing Ki-67, its assessment, and its potential as a prognostic tool in ILC. The most powerful and clinically relevant study to date, mentioned briefly above, is that of Carbognin *et al.*[18] This study investigated the prognostic significance of visually assessed Ki-67 PI in a multicentre series of 875 patients with early stage pure ILC and compared this with Ki-67 PI in patients with IDC. The aims of the study were to identify the best prognostic Ki-67 PI cut-off for disease free survival (DFS) and overall survival (OS) in the two groups and to investigate the impact on long term outcome. The study identified optimal cut-offs for Ki-67 PI in predicting 10 year DFS as $\leq$4% for ILC and $\leq$14% for IDC. In ILC patients the $\leq$4% cut-off was also an independent predictor of 10 year OS. Similar results were obtained in an external validation study of 222 ILCs leading the authors to recommend that, while a higher cut-off value for Ki-67 PI may be appropriate for patients with IDC, a lower Ki-67 value, in the order of 4% or 5%, better stratifies risk in patients with ILC.

In their study of 192 patients with ILC, Narbe *et al.*[6] evaluated Ki-67 proliferation by semi-quantitative visual assessment. Sixty percent of their ILCs stratified as Ki-67 PI low (0–10%), 32% as intermediate (11–30%) and 8% as high (>31%). Low Ki-67 (<30%) identified ILC with a significantly better outcome than tumours with a higher PI in univariate analysis, and a low 'KiGE prognostic index' (grade 1 and grade 2, ER-positive, node negative tumours, with Ki-67 <30%) identified a large group of ILCs with such significantly good long term prognosis it was proposed that chemotherapy could be safely avoided. However, at 30% the Ki-67 cut-off is unlikely to be clinically useful for ILC, as very few cases are likely to be excluded from this good prognosis group. For example, if applied to our cohort, the 30% cut-off only excluded two ILCs (5%) from the 'good prognosis' group.

Wong *et al.*[22] used semi-quantitative scoring of Ki-67 PI to classify tumours as having 'low' (<5%) or 'high' (>10%) proliferation. Approximately 30% of ILCs were found to be 'high' Ki-67 and this was significantly associated with adverse clinicopathologic factors including large tumour size, lymph node involvement and HER2 positivity. There was no discussion of the Ki-67 PI and their proposed cut-off values in relation to outcome or survival data.

In their study of an ILC cohort, Tsai *et al.*[23] compared standard clinicopathological tumour characteristics to the Oncotype Dx Recurrence Score (RS). Discriminant analysis showed that percentage of tumour cells positive for PR, followed by Ki-67 PI had the greatest power in predicting low versus elevated RS, and the authors suggested such observations may provide more widely accessible, faster and less expensive alternatives to Oncotype Dx testing in ILC. However, they did not explore how to apply their observations clinically, with no guidance in regard to statistically significant Ki-67 PI cut-off values.

Given the natural history of ILC is for very long term local and distance recurrence, and the ongoing uncertainty about

the standardisation and clinical utility of Ki-67 PI in ILC, larger studies with sufficient numbers of lobular tumours with long term follow-up are needed to truly establish the value of Ki-67 PI in clinical decision making. That said, the promising data from some of the aforementioned studies is a clear indication that the potential for Ki-67 PI to provide readily available, inexpensive and rapid prognostic information in ILC needs further exploration.

## CONCLUSION

Risk stratification in ILC is absolutely necessary in order to provide personalised and optimised therapies because, despite generally good prognostic clinicopathological parameters, ILC can exhibit long term outcomes akin to higher grade breast carcinomas. Evaluation of Ki-67 PI has great potential in this regard with the power to dichotomise ILC into prognostically divergent groups. In this context, we have demonstrated that automated assessment of the proliferative marker Ki-67 is feasible and accurate in ILC and may represent an affordable, practical and readily available method of ILC risk stratification.

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## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2019.08.004.

**Address for correspondence:** Dr Benjamin Dessauvagie, Division of Pathology and Laboratory Medicine, UWA Medical School, Crawley, WA 6009, Australia. E-mail: ben.dessauvagie@health.wa.gov.au

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