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Consistency in recognizing microinvasion in breast carcinomas is improved by immunohistochemistry for myoepithelial markers

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Abstract

Microinvasion is the smallest morphologically identifiable stage of invasion. Its presence and distinction from in situ carcinoma may have therapeutic implications, and clinical staging also requires the recognition of this phenomenon. Microinvasion is established on the basis of several morphological criteria, which may be difficult and not perfectly reproducible among pathologists. The aim of this study was to assess the consistency of diagnosing microinvasion in the breast on traditional haematoxylin and eosin (HE) stained slides and to evaluate whether immunohistochemistry (IHC) for myoepithelial markers could improve this. Digital images were generated from representative areas of 50 cases stained with HE and IHC for myoepithelial markers. Cases were specifically selected from the spectrum of in situ to microinvasive cancers. Twenty-eight dedicated breast pathologists assessed these cases at different magnifications through a web-based platform in two rounds: first HE only and after a washout period by both HE and IHC. Consistency in the recognition of microinvasion significantly improved with the use of IHC. Concordance rates increased from 0.85 to 0.96, kappa from 0.5 to 0.85, the number of cases with 100 % agreement rose from 9/50 to 25/50 with IHC and the certainty of diagnosis also increased. The use of IHC markedly improves the consistency of identifying microinvasion. This corroborates previous recommendations to use IHC for myoepithelial markers to clarify cases where uncertainty exists about the presence of microinvasion. Microinvasive carcinoma is a rare entity, and seeking a second opinion may avoid overdiagnosis.

Keywords

In situ carcinoma Microinvasion Myoepithelium Immunohistochemistry Reproducibility

Introduction

The European Working Group for Breast Screening Pathology (EWGBSP) was set up in 1993 under the chairmanship of the late Professor John Sloane in order to make the practice of breast pathology more uniform throughout Europe and promote consistent reporting of breast lesions. The members of the EWGBSP are pathologists with a special interest in breast pathology and represent most countries of the European Union where breast screening was introduced. The group was responsible for writing the pathology section of the

European guidelines for quality assurance in breast cancer screening and diagnosis [1] and the Pathology supplements [2]. The supplements include a section on microinvasion, the smallest morphologically identifiable stage of invasion which was introduced as a staging category in 1997 [3].

Microinvasion has been defined in a number of ways. The European guidelines have adopted the most widely accepted definition of one to multiple foci of invasion in the non-specialized interlobular or interductal fibrous or adipose stroma, none larger than 1 mm in greatest dimension. Microinvasion typically occurs with ductal carcinoma in situ (DCIS), but can also occur with lobular carcinoma in situ (LCIS) [2]. The recognition of microinvasive carcinoma and its distinction from in situ carcinoma reflects the ability to detect the first steps of invasion that can be assessed by morphological means. Whether or not this has therapeutic implications, microinvasion remains an important issue in cancer research. Consistent staging also requires the recognition of this phenomenon.

Since recognizing microinvasion may be difficult and not perfectly reproducible among pathologists, the primary aim of the study was to assess the consistency of diagnosing microinvasion on haematoxylin and eosin (HE)-stained slides and to evaluate whether immunohistochemistry (IHC) for myoepithelial markers could improve this.

Material and methods

Members of the EWGBSP were asked to submit cases representing either “in situ” or microinvasive breast carcinomas from their own archives. All representative slides (one HE and one IHC) were sent to Careggi University Hospital, Florence. The set of 50 cases was selected by one of the authors (S.B.) and included 26 diagnosed as “in situ” carcinoma and 24 as microinvasive carcinomas. The cases originated from the breast histological material of the Pathology Departments of the following centres: Careggi University Hospital in Florence (32 cases), Rigshospitalet in Copenhagen (6 cases), the University of Coimbra (4 cases), Donauspital in Wien (3 cases), the Semmelweis University Budapest (2nd Department of Pathology, 2 cases) and the University of Porto, CMP laboratories in Brussels and Bács-Kiskun County Hospital (1 case each). Cases selected had been stained with HE and IHC performed with antibodies against myoepithelial cells (calponin, smooth muscle myosin heavy chain or p63) at the time of reporting.

Digital images, depicting a representative area from each case, were generated using a conventional digital camera (Leica DFC 490) attached to a conventional microscope. Nine images (five HE and four IHC stained for

myoepithelial cells) at different magnifications were captured and saved as jpeg files, which were stored on the website of the Bács-Kiskun County Teaching Hospital referenced below.

The study consisted of two electronic circulations. The instructions for completing the first part of the study, the spreadsheet for reporting the interpretation of the cases and the first set of digital images were uploaded for viewing to https://www.kmk.hu/info/ewgbsp2. In the first circulation, each of the 50 HE-stained cases had 5 jpeg images of objective magnifications ×5, ×10 and ×20 and two at ×40. Two months after completing the first round, a second series of 4 jpeg images representing ×5, ×10, ×20 and ×40 magnifications of relevant areas of the cases stained with IHC for myoepithelium were added to the previous 5 images and uploaded to https://www.kmk.hu/info/ewgbsp3, with modified instructions and a reporting spreadsheet. The case order was changed to reduce potential bias from the first circulation. The cases are stored and can be viewed at the above websites. A self-assessment tool using the same images and the majority opinions has been developed and can be accessed at http://microinvasion.ewgbsp.org

Members of the EWGBSP were asked to report each case independently by completing two different spreadsheets for the two circulations. Contributors evaluated cases using the circulated guidance on “Microinvasive carcinoma” reported in the Supplement to the Fourth Edition of the European Guidelines [2]. Participants were also asked to indicate if they were certain of their classification or not. In the first round, participants were asked to classify the in situ component as DCIS or LCIS. In the second round, members assessed only the presence or absence of microinvasion and indicated their certainty of diagnosis.

Kappa values were calculated according to Fleiss [4] for the classification of the in situ neoplasia and for microinvasion. Further comparisons used the chi-square test according to Yates, corrected for continuity with the VassarStats software (Vassar College, Poughkeepsie, NY) [5].

Results

Twenty-eight members of the EWGBSP responded to the call to take part in the study. The main results are summarized in Tables 1 and 2 plus Figs. 1 and 2.

Table 1

Majority classification of the cases as microinvasive or in situ in rounds 1 and 2 of the study

Round 2 labels	Microinvasion present? Round 1 majority classification	Round 1 majority	Microinvasion present? Round 2 majority classification	Round 2 majority	Round 2 labels	Microinvasion present? Round 1 majority classification	Round 1 majority	Microinvasion present? Round 2 majority classification	Round 2 majority
Case 1	N	1.00	N	1.00	Case 26	Y	0.82	Y	1.00
Case 2	N	1.00	N	1.00	Case 27	N	1.00	N	1.00
Case 3	Y or N	0.50	Y	0.96	Case 28	Y	0.89	Y	0.96
Case 4	Y	1.00	Y	0.96	Case 29	N	0.82	N	1.00
Case 5	Y	0.89	Y	1.00	Case 30	N	0.75	N	1.00
Case 6	N	0.89	N	1.00	Case 31	N	0.75	Y	0.71
Case 7	N	0.86	N	1.00	Case 32	Y	0.68	Y	0.82
Case 8	Y	0.75	Y	0.96	Case 33	N	0.86	N	1.00
Case 9	Y	0.54	Y	0.89	Case 34	Y	0.86	Y	0.96
Case 10	N	0.96	N	1.00	Case 35	N	0.79	Y	0.96
Case 11	Y	0.96	Y	1.00	Case 36	Y	0.57	Y	0.89
Case 12	N	0.75	N	1.00	Case 37	N	0.93	N	1.00
Case 13	N	1.00	N	1.00	Case 38	Y	0.82	Y	0.93
Case 14	N	0.96	N	1.00	Case 39	N	1.00	N	1.00
Case 15	Y	0.96	Y	0.96	Case 40	N	0.79	N	1.00
Case 16	Y	0.54	N	0.93	Case 41	N	0.82	N	1.00
Case 17	Y	0.75	Y	0.89	Case 42	N	0.82	N	0.96
Case 18	N	1.00	N	0.93	Case 43	Y	0.93	Y	1.00
Case 19	Y	0.86	Y	0.96	Case 44	Y	0.82	Y	0.96
Case 20	N	1.00	N	1.00	Case 45	N	0.89	N	0.96
Case 21	Y	0.96	Y	1.00	Case 46	N	0.89	N	0.93
Case 22	Y	0.71	N	0.75	Case 47	N	0.57	N	1.00
Case 23	N	0.93	N	1.00	Case 48	N	1.00	N	0.96

Table 2
Consistency of diagnosing different aspects of the 50 lesions studied

	Presence of microinvasion (round 1)	Type of background in situ neoplasia	Presence of microinvasion (round 2)
Majority opinions based on an average rate	0.84	0.96	0.96
Majority opinion ranges	0.50–1.00	0.70–1.00	0.71–1.00
Observers' matching the majority opinion range	0.72–0.94	0.84–1.00	0.80–1.00
Cases with 100 % agreement (%)	9 (18 %)	34 (68 %)	25 (50 %)
Overall kappa values	0.50	0.65	0.85

Fig. 1
Concordance rates according to majority diagnoses in the first circulation. MIC microinvasion, CIS carcinoma in situ (DCIS or LCIS), R1 round

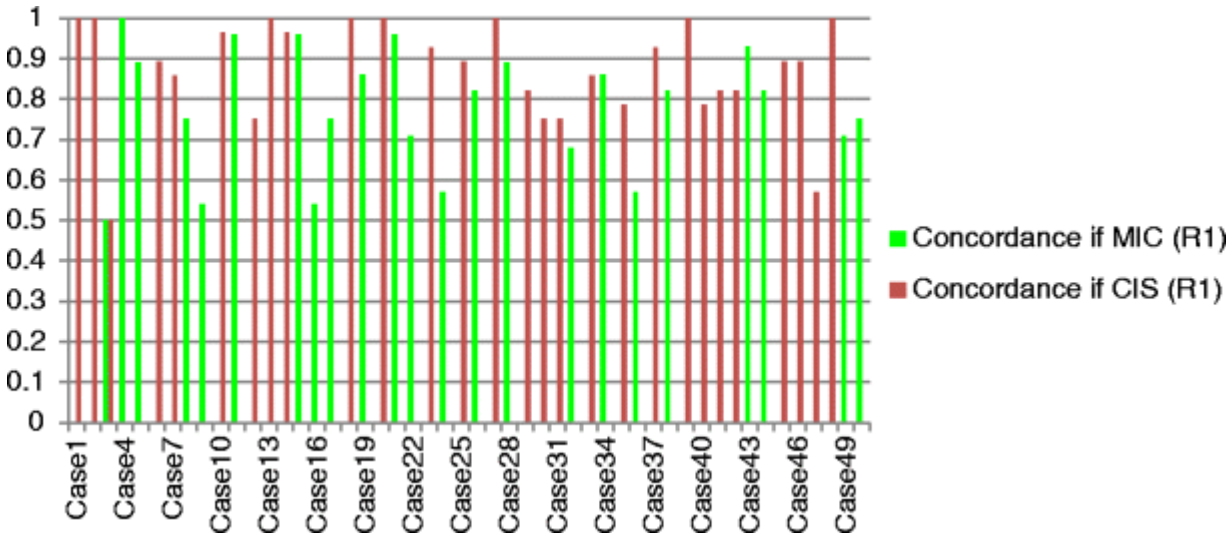
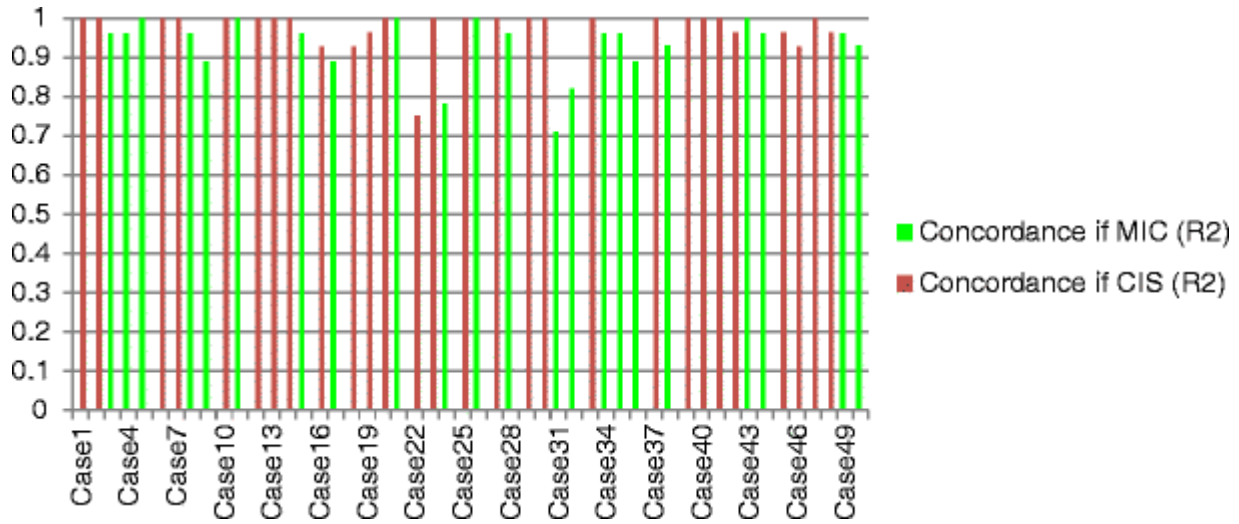


Fig. 2
Concordance rates according to majority diagnoses in the second circulation. MIC microinvasion, CIS carcinoma in situ (DCIS or LCIS), R2 round 2



Consistency in the recognition of microinvasion was significantly improved with the use of IHC (circulation 2) compared with the diagnosis on HE alone (circulation 1). In the first round, only nine cases reached 100 % consensus in diagnosis with eight cases reported as in situ and one as microinvasion. This changed to 20 and 5, respectively, in round 2, meaning that half of the cases had 100 % agreement in diagnosis (Tables 1 and 2). The concordance rates with the majority diagnosis were 0.85 (1187 ratings out of all 1400 ratings) and 0.96 (1343/1400) for circulations 1 and 2, respectively. The kappa coefficient of 0.5 reflecting moderate reproducibility on the basis of HE-stained slides increased to 0.85 with the addition of IHC, reflecting almost perfect reproducibility [6]. Uncertainty was recorded with higher frequency in the first round than in the second (mean 34.5 %; range 8–84 versus 9.7 %; range 0–38 %; $p < 0.0001$, Yates chi-square).

One case in round 1 (Fig. 3, Table 1) was inconclusive, with half of the observers for and the other half against microinvasion; this case was diagnosed as microinvasion by all but one participant in the second circulation. Two cases converted from in situ to microinvasion in round 2 on the basis of majority opinion (Fig. 4, Table 1). In these two cases, 5 and 7 observers would have asked for IHC in the first round to support or refute their opinion. Two cases converted the opposite way (Fig. 5, Table 1); here six and four participants would have asked for IHC. The remaining cases were identically diagnosed by the majority in the two circulations.

Fig. 3
Case 3 (round 2) with corresponding images from rounds 1 and 2. The case was diagnosed as microinvasive by half of the observers. Stains and objective magnification—a HE ×5, b HE ×10, c HE ×20, d IHC ×40

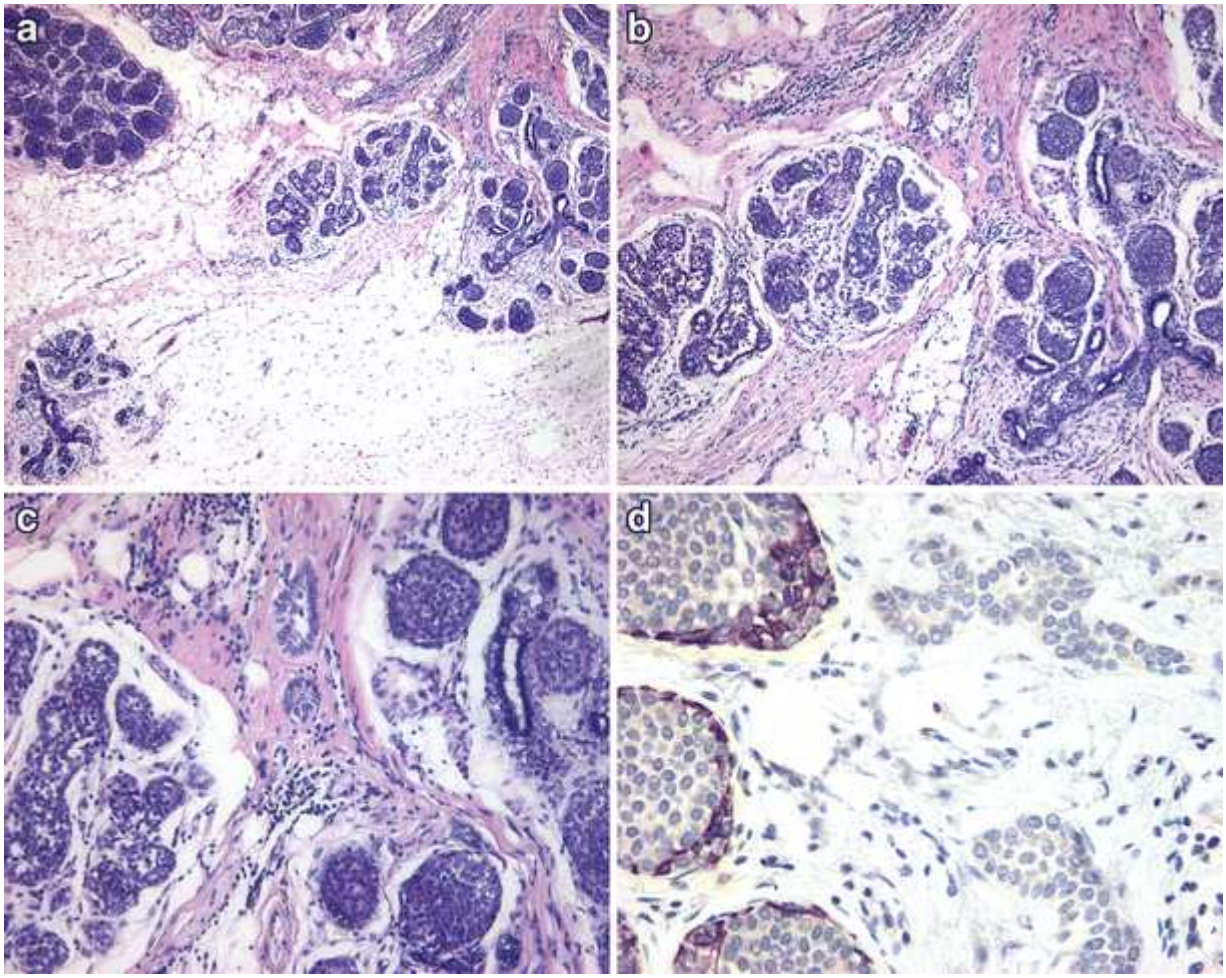


Fig. 4
The two cases converting from negative for microinvasion to positive for it. a, b Case 31 (round 2) HE ×20, IHC ×20, respectively; c, d Case 35 (round 2) HE ×20, IHC ×20, respectively

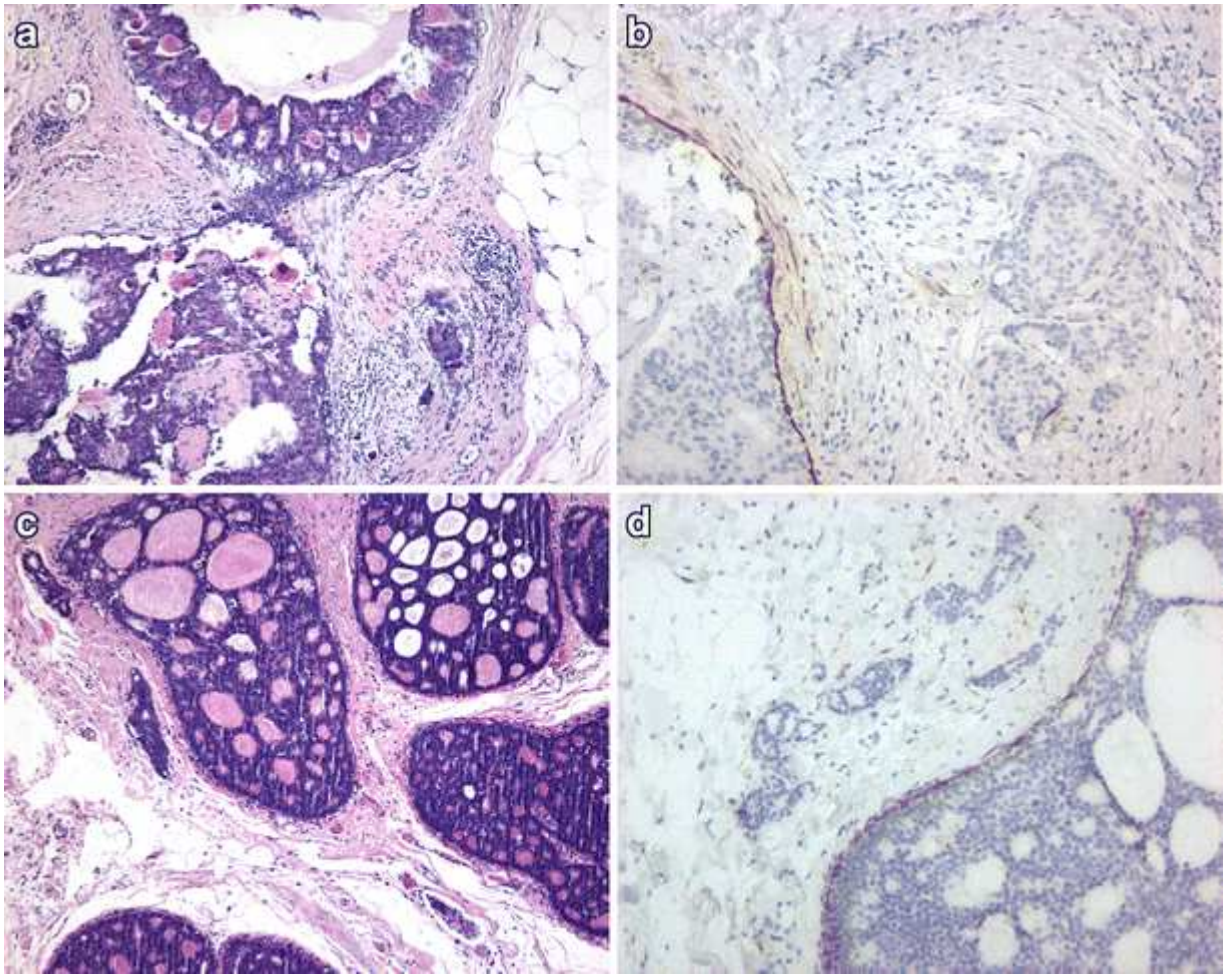


Fig. 5
The two cases converting from positive for microinvasion to negative for it. a, b Case 16 (round 2) HE ×20, IHC ×20, respectively; c, d Case 22 (round 2) HE ×20, IHC ×20, respectively. The small cluster of cells (arrowhead) in the specialized stroma might have been responsible for the change in classification in case 22.

For cases diagnosed as discrepant from the majority diagnosis, the observers were uncertain of their classification in the majority of these (Figs. 6 and 7). The recorded certainty of observers in cases discrepant from the majority diagnosis was 0.36 (79/218) and 0.45 (26/58) in rounds 1 and 2, respectively ($p = 0.30$, Yates chi-square). Comparing Figs. 6 and 7, the consistency improved, but highlights a higher degree of observer certainty for the fewer cases diagnosed as discrepant.

Fig. 6
Numbers of deviations from majority opinion with indication of certainty in round 1. Obs observer. Note the difference in scale from Fig. 7

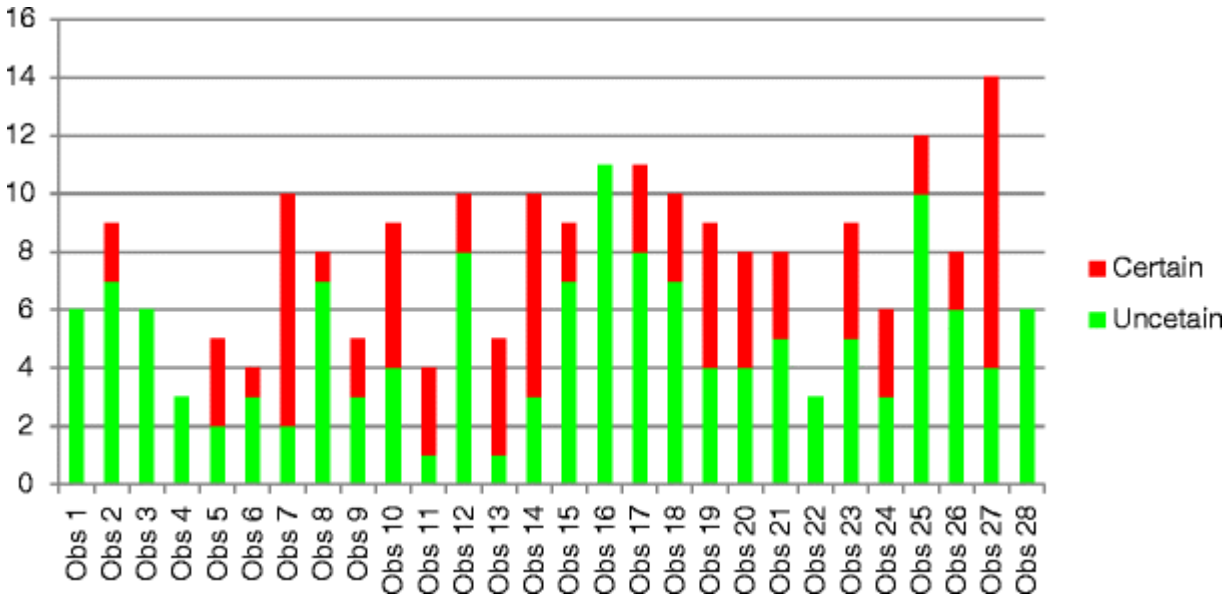
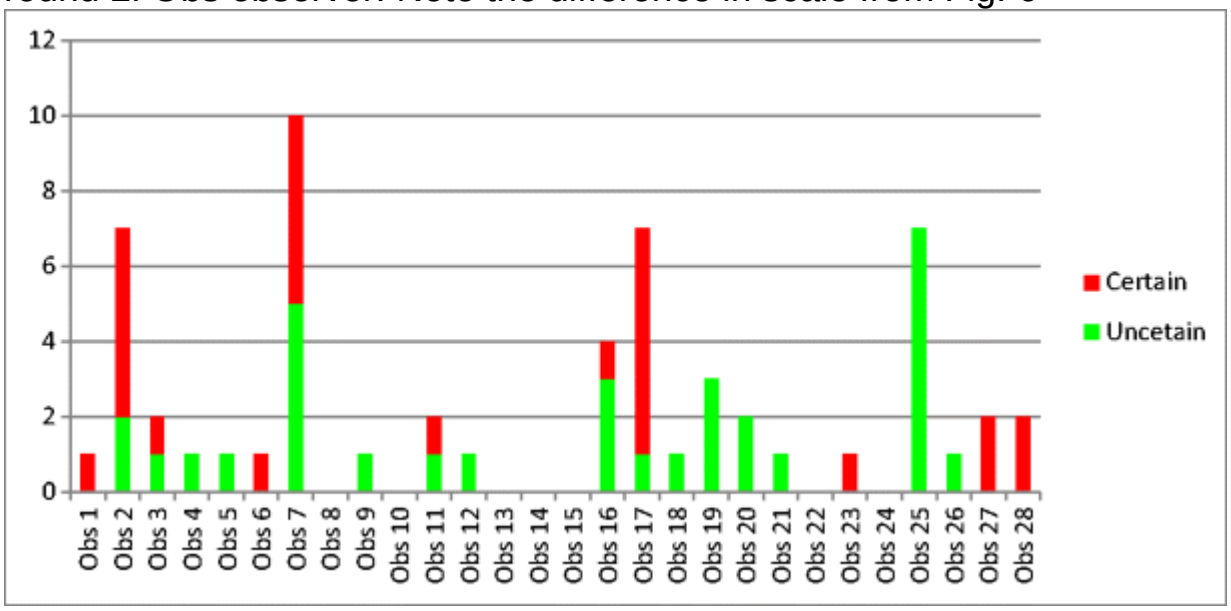


Fig. 7
Numbers of deviations from majority opinion with indication of certainty in round 2. Obs observer. Note the difference in scale from Fig. 6



Regarding the classification of the in situ carcinoma component, the majority diagnosis was DCIS in 46 cases and LCIS in 4 cases with a mean concordance of 0.96 (range 0.70–1.00). Thirty-four of the 50 cases were unanimously classified. Several participants would have added e-cadherin immunohistochemistry to support their classification. The overall kappa value and the kappa values of the individual classes of DCIS or LCIS were all 0.65, reflecting substantial interobserver agreement [6]. All cases of LCIS were of classical type. No pleomorphic LCIS or atypical lobular hyperplasia were represented in this series. Of the DCIS lesions 24, 18 and 4 were graded as of high, intermediate and low grade, respectively.

Discussion

Currently, the surgical treatment strategy for in situ and microinvasive carcinoma is the same in most institutions [7, 8], but finding a focus of invasion consistent with microinvasion in a preoperative needle biopsy specimen would lead to an indication for sentinel node biopsy in some units. Therefore, the recognition of microinvasion has a value. It also helps to analyse the earliest changes associated with morphologically identifiable invasion, and some systemic treatment guidelines recognize this entity as a potential indication for treatment according to early invasive carcinoma protocols [9], although this is not a uniform approach.

The distinction between in situ and invasive carcinoma depends on the recognition of a disorganized infiltrative growth pattern (e.g. single cells, distorted tubules or nests), desmoplasia and a tissue reaction (e.g. an inflammatory response), as well as the lack of a myoepithelial layer and a basement membrane in invasive cancer. In general, most of these features occur together, but there are cases where only some of them are present, making the distinction less obvious. For example, some well-differentiated invasive carcinomas display an abortive basement membrane [10–12] and lack a desmoplastic or inflammatory tissue reaction. At the other end of the spectrum, some in situ carcinomas also demonstrate some of these alterations; the myoepithelium in some cases of DCIS may be atrophic and barely visible, may have an altered phenotype and may lack the expression of some myoepithelial markers [13]. DCIS may also be associated with architectural distortion of the involved breast tissue and with significant periductal or periacinar inflammation and fibrosis. These features make the recognition of microinvasion difficult [14].

The consistency of diagnosis depends on many factors, including the criteria used, the experience and attentiveness of the reporting pathologist, the relative sizes of the material and the feature to be recognized, the typical or atypical nature of the lesion and the use of special techniques. The diagnostic criteria formulated by the EWGBSP in the last Supplement to the European guidelines [1, 2], which correspond to those of the UK guidelines [15], were used in this study. The lesions of interest were represented in digital images of various and sequential magnifications, reducing the possibility of missing small lesions. The participants were pathologists of many years of experience with a special interest in breast pathology. As the artificial conditions of the study were nearly ideal to allow good reproducibility, it is surprising that the interobserver agreement in recognizing microinvasion was only moderate on the HE-stained slides (kappa = 0.5). As the original areas were diagnosed as

in situ carcinoma or microinvasive in nearly equal proportions, it is also obvious that consistency is better in excluding microinvasion than confirming it. The use of myoepithelial cell markers resulted in a marked improvement in the consistency of reporting microinvasion, although full agreement was more often reached for cases reported as in situ carcinoma.

On the basis of the presented results, it seems obvious that IHC for myoepithelial markers should be recommended in cases of doubt about the presence or absence of microinvasion. However, some comments must be made. Firstly, pathologists should be aware of the fact that the lack of staining with a single myoepithelial marker is not sufficient to exclude the presence of a myoepithelial cell layer as phenotypical changes may lead to the absence of staining with some antibodies even in the presence of myoepithelium [13, 16–18]. In this respect, there seems to be no ideal marker, and doubtful cases should be assessed by two to three antibodies in parallel. Secondly, by consensus and definition, invasion of the specialized (intralobular or periductal) stroma does not make the diagnosis of microinvasion acceptable. Although the participants were aware of the definitions, several comments were made about the invasion of the specialized stroma (e.g. in case 22 according to round 2 labelling, Fig. 3c, d). The significance of such minute foci of invasion into the intralobular stroma is unknown from the theoretical point of early invasion-associated changes, but from a pragmatic aspect, this phenomenon seems negligible from the outcome point of view. Guidelines have recommendations on how to deal with uncertainty in identifying staging categories: they suggest to opt for the lower stage category, i.e. lack of microinvasion in this setting [15, 19]. Thirdly, a wrong categorization may be made with certainty and conviction. Concentrating on the cases which were classified against the majority, the participants were sometimes certain of their classifications, although uncertainty predominated in these cases. The use of IHC reduced the overall proportion of uncertainly classified cases, but microinvasion or its lack was diagnosed with certainty in a higher proportion of “misclassified” cases (0.45 versus 0.36). Finally, it must also be remembered that pathological diagnosis is not a democratic process, as one of the authors has highlighted during a teaching course, and majority diagnoses are not necessarily right. However, a majority classification was considered the gold standard in this study as it makes it more probable that second, third or further opinions would match and would result in this diagnosis. In light of these considerations, the discrepancy between the original diagnosis and the majority diagnosis seems explainable. Originally, 26 cases were reported as non-microinvasive, but the majority diagnosis used as the gold standard here identified 27 cases as in situ carcinoma.

As microinvasion can occur with in situ carcinomas of both lobular (LCIS) and ductal (DCIS) types, the study also looked at the reproducibility of the classification of the background lesions. The distinction between LCIS and DCIS was good with the majority opinions averaging 94 % (Table 2).

Analysis of interobserver agreement in the classification of invasive breast carcinoma and its prognostic indices has demonstrated only moderate reproducibility [20, 21]. There is a paucity of information in the literature on interobserver agreement in the diagnosis of microinvasion. The microfocal nature of the process renders it unsuitable for evaluation in external quality assurance schemes, many of which utilize glass slides. In a series of 870 screen-detected breast cancers reviewed by 3 British pathologists with specialist expertise in breast pathology, only 3 of 17 cases, categorized as microinvasive carcinoma by any of the participants, achieved full observer agreement [22]. In the quoted study, 4 of the 17 cases were classified as microinvasive by 2 pathologists, and the remaining 10 cases by only one, therefore resulting in a non-invasive carcinoma majority diagnosis in these latter 10 [22]. This means that 37 out of 51 opinions (0.73) on these 17 cases were concordant in the diagnosis of microinvasive carcinoma or in the lack of microinvasion; this can be compared with the 0.84 concordance rate reached in round 1 of the current study and 0.96 in round 2. A review of a large series of consultation cases reported a concordance rate of 0.7 between general pathologists and specialist breast pathologists in the diagnosis of 5 cases of DCIS with microinvasion [23]. In contrast to the current study, these studies included small numbers of cases of microinvasion and diagnosis was based on evaluation of HE-stained slides only.

Finally, some limitations of the present study must be mentioned. The cases were neither a consecutive series nor were they randomly selected; rather, they were selected after a review of the original pathology reports. Most cases were derived from one institution although cases from other sources were included because of the rarity of microinvasive carcinoma. Therefore, it could be argued that one cannot make generalizations based on our results, as the data pertain to the evaluation of a specific series of cases by those pathologists who participated in the study. However, microinvasive carcinoma is rare and it is very likely that most cases in which microinvasion was either diagnosed or entered into the differential diagnosis in the relevant institutions were included. We also feel strongly that a minor selection bias would not influence the major conclusion of the study, namely, that IHC improves the consistency in diagnosis. Another limitation of the study is that the pathologists examined images of preselected areas and not the whole slide

or all the slides from a given specimen. Therefore, any data on consistency would be expected to be worse in real life. But again, this does not substantially influence the conclusion that, whenever there is doubt, the demonstration of myoepithelium by IHC helps to clarify the diagnosis.

In summary, the results presented here corroborate previous recommendations to use IHC for myoepithelial markers to clarify cases where uncertainty exists about the presence of microinvasion [24]. In the study settings, this resulted in marked improvement of the consistency of identifying microinvasive cases. Overall, microinvasive carcinoma is a rare entity and seeking a second opinion may avoid overdiagnosis.

Acknowledgments

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no competing interests.

References

1. Amendoeira I, Apostolikas N, Bellocq JP, et al. (2006) Quality assurance guidelines for pathology. In: Perry N, Broeders M, de Wolf C, et al. (eds) European guidelines for quality assurance in breast cancer screening and diagnosis, 4th edn. European Comission, Luxembourg, pp. 219–311

2. Wells CA, Amendoeira I, Bellocq JP, et al (2012) S2: Pathology update. Quality assurance guidelines for pathology. In: Perry N, Broeders M, de Wolf C, et al (eds) European guidelines for quality assurance in breast cancer screening and diagnosis, 4th ed, Supplements. European Commission, Office for Official Publications of the European Union, Luxembourg, pp 73–120

3. Sobin LH, Wittekind CH (1997) UICC TNM classification of malignant tumours, 5th edn. Wiley-Liss, New York

4. Fleiss JL (1981) The measurement of interrater agreement. In: JL F (ed) Statistical methods for rates and proportions, 2nd ed. John Wiley and Sons, New York, pp. 212–236

5. Richard Lowry (2015)

6. Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. Biometrics 33:159–174

7. Gojon H, Fawunmi D, Valachis A (2014) Sentinel lymph node biopsy in patients with microinvasivebreast cancer: a systematic review and meta-analysis. Eur J Surg Oncol 40:5–11

8. Hanna MG, Jaffer S, Bleiweiss IJ, et al. (2014) Re-evaluating the role of sentinel lymph node biopsy in microinvasive breast carcinoma. Mod Pathol 27:1489–1498

9. NCCN clinical practice guidelines in oncology—breast cancer v2–2015.

10. Gusterson BA, Warburton MJ, Mitchell D, et al. (1982) Distribution of myoepithelial cells and basement membrane proteins in the normal breast and in benign and malignant breast diseases. Cancer Res 42:4763–4770

11. Nerlich AG, Wiest I, Wagner E, et al. (1997) Gene expression and protein deposition of major basement membrane components and TGF-beta in human breast cancer. Anticancer Res 17:4443–4449

12. Cserni G (2008) Presence of basement membrane material around the tubules of tubulolobular carcinoma. Breast Care 3:423–425

13. Hilson JB, Schnitt SJ, Collins LC (2009) Phenotypic alterations in ductal carcinoma in situ-associated myoepithelial cells: biologic and diagnostic implications. Am J Surg Pathol 33:227–232

14. Bianchi S, Vezzosi V (2008) Microinvasive carcinoma of the breast. Pathol Oncol Res 14:105–111

15. Ellis IO, Pinder SE, Bobrow L, et al (2005) Pathology reporting of breast diseases. NHSBSP Publication No 58 January 2005

16. Hilson JB, Schnitt SJ, Collins LC (2010) Phenotypic alterations in myoepithelial cells associated with benign sclerosing lesions of the breast. Am J Surg Pathol 34:896–900

17. Cserni G (2012) Benign apocrine papillary lesions of the breast lacking or virtually lacking myoepithelial cells—potential pitfalls in diagnosing malignancy. Apmis 120:249–252

18. Kővári B, Szász AM, Kulka J, et al. (2015) Evaluation of p40 as a myoepithelial marker in different breast lesions. Pathobiology 82:165–170

19. Sobin LH, Gospodarowicz MK, Wittekind C (2010) UICC TNM classification of malignant tumours, 7th edn. Wiley-Blackwell, Chichester

20. Ellis IO, Coleman D, Wells C, et al. (2006) Impact of a national external quality assessment scheme for breast pathology in the UK. J Clin Pathol 59:138–145

21. Parham DM, Coleman D, Kodikara S, et al. (2006) The NHS breast screening programme (pathology) EQA: experience in recent years relating to issues involved in individual performance appraisal. J Clin Pathol 59:130–137

22. Anderson TJ, Sufi F, Ellis IO, et al. (2002) Implications of pathologist concordance for breast cancer assessments in mammography screening from age 40 years. Hum Pathol 33:365–371

23. Gomes DS, Porto SS, Balabram D, et al. (2014) Inter-observer variability between general pathologists and a specialist in breast pathology in the diagnosis of lobular neoplasia, columnar cell lesions, atypical ductal hyperplasia and ductal carcinoma in situ of the breast. Diagn Pathol 9:121

24. Damiani S, Ludvikova M, Tomasic G, et al. (1999) Myoepithelial cells and basal lamina in poorly differentiated in situ duct carcinoma of the breast. An immunocytochemical study. Virchows Arch 434:227–234