Virchows Arch (2017) 471:3–12
DOI 10.1007/s00428-017-2147-6

ORIGINAL ARTICLE

Invasive lobular carcinoma with extracellular mucin production—a novel pattern of lobular carcinomas of the breast. Clinico-pathological description of eight cases

Gábor Cserni[1,2] · Giuseppe Floris[3,4] · Nektarios Koufopoulos[5] · Anikó Kovács[6] · Afroditi Nonni[7] · Peter Regitnig[8] · Anders Stahls[9] · Zsuzsanna Varga[10]

Received: 6 March 2017 / Revised: 2 May 2017 / Accepted: 8 May 2017 / Published online: 20 May 2017

Abstract Invasive lobular carcinoma of the breast is known to produce intracellular mucin and has been recognized in single-case reports to show extracellular mucin production, as well. This latter morphology is not only rare but must also be under- or misdiagnosed. The aim was to better characterize this entity. Cases of lobular cancers demonstrating extracellular mucin formation were identified in a multi-institutional effort and their clinical and morphologic features were assessed. Immunohistochemistry was used to characterize the E-cadherin-membrane complex, neuroendocrine differentiation, and to some extent, mucin formation. All but one of the eight cases occurred in postmenopausal patients. Extracellular mucin production was present in 5 to 50% of the tumour samples and rarely also appeared in nodal and distant metastases. The tumours were completely E-cadherin negative and showed cytoplasmic p120 positivity. The majority (n = 6/8) was also completely negative for β-catenin, but two tumours displayed focal β-catenin positivity in the mucinous area. MUC1 and MUC2 expression was observed in all and 7/8 tumours, respectively; neuroendocrine differentiation was present in only one. Invasive lobular carcinoma with extracellular mucin formation is a rare morphologic variant of lobular carcinoma prone to be misdiagnosed and warranting further studies.

Keywords Breast cancer · E-cadherin · Extracellular mucin · Lobular carcinoma · Mucinous carcinoma

Electronic supplementary material The online version of this article (doi:10.1007/s00428-017-2147-6) contains supplementary material, which is available to authorized users.

✉ Gábor Cserni
cserni@freemail.hu

[1] Department of Pathology, Bács-Kiskun County Teaching Hospital, Nyíri út 38, Kecskemét 6000, Hungary

[2] Department of Pathology, University of Szeged, Állomás u. 1, Szeged 6725, Hungary

[3] Department of Imaging and Pathology, Laboratory of Translational Cell and Tissue Research, KU Leuven-University of Leuven, Herestraat 49, 3000 Leuven, Belgium

[4] Department of Pathology, KU Leuven-University of Leuven, University Hospitals Leuven , Herestraat 49, 3000 Leuven, Belgium

[5] Department of Pathology, "Saint Savvas" Anticancer Hospital of Athens, 171 Alexandras av, 115 22 Athens, Greece

[6] Department of Clinical Pathology and Genetics, Sahlgrenska University Hospital, Gula stråket 8, 41 345 Gothenburg, Sweden

[7] First Department of Pathology, Medical School, National and Kapodistrian University of Athens, Mikras Asias Str. 75, 115 27 Athens, Greece

[8] Institute of Pathology, Medical University of Graz, Auenbruggerplatz 25, 8036 Graz, Austria

[9] Department of Pathology, University of Helsinki and HUSLAB, Helsinki University Hospital, P.O. Box 400, 00029 Helsinki, Finland

[10] Department of Pathology and Molecular Pathology, University Hospital Zurich, Schmelzbergstrasse 12, 8091 Zürich, Switzerland

Introduction

Invasive lobular carcinoma (ILC) is the most common special type of breast carcinoma characterized by peculiar morphology, molecular background, clinical manifestation, including radiologic presentation and metastatic pattern. Besides classical ILC described by Foote and Stewart [1], several variant forms have been reported in the literature, including solid [2], signet ring cell [3], tubulolobular [4], alveolar [5], trabecular [5], and pleomorphic [6–8] variants. ILC of mixed type consists of a mixture of the classic type with one or some of the other subtypes without quantitative requirement from the part of the components [9, 10]. Variant patterns often present as part of ILC mixed type, and their pure form is rather rare.

Current classifications define ILC with its morphological features and include the loss of E-cadherin expression and alterations in other E-cadherin complex molecules like β-catenin and p120-catenin as characteristic [10, 11]. The categorization of lobular carcinoma as lobular on morphological grounds alone is not always straight forward, and inter-observer variation has been described [12]. The recognition of different variants of the disease can also pose difficulties. E-cadherin immunohistochemistry (IHC) may aid in the distinction from other tumour types [13], but requires also careful evaluation of the staining and its context. Non-lobular carcinomas have also been described to show diminished or lost E-cadherin expression [14, 15], and aberrant E-cadherin expression has been a noticed phenomenon in a subset of ILCs [16]. However, in non-lobular carcinomas with reduced or lacking E-cadherin expression, the integrity of the E-cadherin-membrane complex seems maintained as illustrated by the membranous expression of β- and p120-catenins [17]. In contrast, aberrant expression of E-cadherin seen in lobular carcinomas is associated with the loss of membranous staining for other molecules of the E-cadherin-membrane complex [18]. Therefore, the analysis of E-cadherin, β-catenin, and p120-catenin may help to better classify tumours with non-obvious morphological features of ILC.

Some of the authors of this manuscript have encountered a variant of ILC with extracellular mucin production (ILC-ECMUC) partly reminiscent of mucinous carcinoma that has not yet been included in the pattern classification of ILC [9–11]. A few previous single-case reports suggest that others had also recognized this entity before [19–23]. A series of such cases has been collected from archived routine and consultation material of the members of the European Working Group for Breast Screening Pathology in order to characterize this form of ILC.

Materials and methods

The authors have searched their files for identifying cases that might probably represent ILC-ECMUC. Representative formalin-fixed paraffin-embedded blocks from the material used for the original diagnostic work-up were selected and were analysed for the expression of the antigens highlighted by the antibodies listed in Table 1. Whenever IHC was done at the local laboratory either as a diagnostic aid or as a confirmation of the case matching ILC-ECMUC, the results were taken into account and IHC was not repeated. Whenever a stain was missing from the panel, a formalin-fixed and paraffin-embedded block or unstained slides were sent to the first author's laboratory for IHC to be done. It ensues that the antibodies used were not uniform as highlighted in Table 1. The authors agreed that although there might be differences between the antibodies, in general practice, all of them are used in the diagnostic setting and are suitable to make a diagnosis based on the presence or absence of staining and the localization/pattern of staining. Therefore, no attempt was made to harmonize the clones used. In addition to IHC, periodic acid Schiff (PAS) and PAS-Alcian blue stains (at pH 2.5) were obtained from the cases.

For oestrogen and progesterone receptors (ER and PR), as well as for human epidermal growth factor receptor 2 (HER2), the American Society of Clinical Oncology/College of American Pathologists recommendations were used for classifying the tumours as positive or negative [24, 25]. For other immunostains, the pattern was considered diffuse when ≥50% of the cells stained, and focal when <50% showed staining; complete lack of staining was considered negative. Multifocality was defined according to Tot, i.e. distinct invasive tumour foci separated by any amount of uninvolved breast tissue [26].

The ethical committee of Bács-Kiskun County Teaching Hospital was consulted about this retrospective non-interventional study and no objections were raised. The approval (no 1/16 10 26) included a waiver of an informed consent in this study setting. As mandated by local regulations, the data safety manager of Bács-Kiskun County Teaching Hospital also approved the study, and the case from Zurich was also covered by a local ethical approval of the Canton Zurich.

Results

The present series includes eight ILC-ECMUCs seen between 1998 and 2016 and retrieved from seven European pathology institutions. All but one of these tumours were diagnosed in postmenopausal women, of whom five had no hormonal replacement therapy (HRT), whereas two had no information available in this context. The mean age of the patients was 62.9 (range 45–75) years. The reported cancers were all palpable at presentation, the mammographic appearance was available for seven of them: five presented as masses (spiculated or nodular, two associated with microcalcifications) and two showed only architectural distortion. Seven tumours

Table 1 Antibodies used in the series

	Clone	Source	Dilution	Number of cases stained
ER	6F11	Leica/Novocastra, Newcastle, UK	1:50	4
	1D5	Dako, Glostrup, Denmark	1:50	1
	EP1	Dako, Glostrup, Denmark	RTU	1
	SP1	Roche/Ventana, Tucson, AZ	RTU	2
PR	Pgr312	Leica/Novocastra, Newcastle, UK	1:50–1:200	5
	Pgr636	Dako, Glostrup, Denmark	RTU	2
	1E2	Roche/Ventana, Tucson, AZ	RTU	1
HER2	4B5	Roche/Ventana, Tucson, AZ	RTU	6
	HercepTest	Dako, Glostrup, Denmark	RTU	1
	Polyclonal (A0485)	Dako, Glostrup, Denmark	1:300	1
E-cadherin	36B5	Leica/Novocastra, Newcastle, UK	1:40	4
	36	Roche/Ventana, Tucson, AZ	RTU	1
	EP700Y	Cell Marque, Rocklin, CA	1:200	1
	HECD-1	Zymed/Invitrogen	1:200	1
	NCH-38	Dako, Glostrup, Denmark	RTU	1
Beta-catenin	Clone 14	Histopathology, Pécs, Hungary	1:150	5
	Clone 14	Roche/Ventana, Tucson, AZ	RTU	1
	Clone 14	BD Bioscience, San Jose, CA, USA	1:50	1
	CAT-5H10	Zymed/Invitrogen	1:400	1
p120	98/pp120	Biocare, Concord, CA	1:150	6
	Clone 98	BD Bioscience, San Jose, CA, USA	1:200–300	2
HMWCK	34beta-E12	Cell Marque, Rocklin, CA	1:300	1
		Dako, Glostrup, Denmark	RTU, 1:50, 1:250	6
MUC1 (EMA)	E29	Biogenex, Fremont, CA	1:100	1
	EPR1023	Biogenex, Fremont, CA	RTU	4
	MA552	Leica/Novocastra, Newcastle, UK	1:100	1
	MA695	Leica/Novocastra, Newcastle, UK	1:50	1
	MRQ17	Roche/Ventana, Tucson, AZ	RTU	1
MUC2	MRQ18	Cell Marque, Rocklin, CA	1:200	5
	Ccp58	Leica/Novocastra, Newcastle, UK	1:100	2
	Ccp58	Scy Tec, Logan, UT, USA	1:10	1
Chromogranin	LK2H10	Cell Marque, Rocklin, CA	RTU	5
	LK2H10-phe5	Thermo Fisher Scientific, Waltham, MA	1:3000	1
	DAK-A3	Dako, Glostrup, Denmark	1:50	1
	Polyclonal (A0430)	Dako, Glostrup, Denmark	1:800	1
Synaptophysin	Polyclonal	Cell Marque, Rocklin, CA	RTU	1
	MRQ40	Cell Marque, Rocklin, CA	1:200	1
	27G12	Leica/Novocastra, Newcastle, UK	RTU, 1:20	4
	SP11	Roche/Ventana, Tucson, AZ	RTU	1
	DAK-SYNAP	Dako, Glostrup, Denmark	1:50	1
CD56 (NCAM)	123C3.D5	Cell Marque, Rocklin, CA	1:50, 1:400	5
	123C3	Roche/Ventana, Tucson, AZ	RTU, 1:50	2
	MRQ-42	Cell Marque, Rocklin, CA	1:500	1
Ki67	MIB 1	Dako, Glostrup, Denmark	RTU, 1:100	7
	Clone 30.09	Roche/Ventana, Tucson, AZ	RTU	1

ER oestrogen receptor, *EMA* epithelial membrane antigen, *HER2* human epidermal growth factor receptor 2, *HMWCK* high-molecular weight cytokeratin, *NCAM* neural cell adhesion molecule, *PR* progesterone receptor, *RTU* ready to use (prediluted)

had a core needle biopsy preoperatively: three were originally and/or preoperatively diagnosed as having a mucinous carcinoma component without the recognition that this represented part of ILC (cases 1, 2, and 4); two had the diagnosis of lobular carcinoma and no mucin production was visualized even in retrospect (cases 5 and 8); and two were recognized as representing ILC-ECMUC (cases 3 and 7). Some of the characteristics of the tumours are summarized in Table 2.

Of the eight cases analysed, seven were from surgically resected tumours, and the eighth was a core needle biopsy. All tumours demonstrated areas of non-mucinous ILC and five of the eight cases also included foci of classic lobular

neoplasia (LN), a term with a meaning encompassing lobular carcinoma in situ, atypical lobular hyperplasia, and ductal involvement by the same type of neoplastic cells with or without pagetoid spread [10]; a component of more pleomorphic LN was identified in case 1. LN showed production of extracellular mucin in two cases (Fig. 1). The non-mucinous ILC component was of the classical type in seven cases, along with solid-type ILC in three cases, and there was only a solid component in one case. The mucinous area represented about 10 to 70% of the tumour in resection specimens, and 5% of the core biopsy, where it formed a single nodule of 0.6 mm in diameter. In this latter case (case 7), neoadjuvant chemotherapy was administered and mastectomy was performed afterwards. The review of the mastectomy specimen showed no remaining extracellular mucinous component, but had about 30% of pleomorphic ILC component. Microscopic multifocality was identified in three cases. Case 8 displayed a focal area of necrosis.

Extracellular mucin production was seen either in the form of relatively circumscribed single or multiple nodular areas (7/8 cases) or was patchy with irregular borders through different areas of the tumour (1/8 cases) (Fig. 1a–c). One case featured circumscribed foci of mucin production and smaller areas with irregular lakes of this substance, and another case had a nodular area of mucin production with partly irregular borders. The mucinous component stained with PAS and was blue with the combined PAS-Alcian blue stain.

Tumour cells in the mucinous area were often arranged in clusters or even simple or cribriform gland-like structures, although cellular cohesion (in contrast to glands in mucinous carcinoma) was not obvious and some dissociated single cells were generally present (Fig. 2). One case showed no clustering of the cells, although some Indian filing, a feature typical of classical lobular carcinoma was identified in it (Fig. 2a). The cells displayed moderate nuclear pleomorphism. Higher-grade nuclei were identified only in a minor focus of LN in case 1 and in about 30% of the non-mucinous part in the mastectomy specimen of case 7, in which the mucinous component was present only in the core needle biopsy. Local recurrence of case 4 also displayed pleomorphic features.

Signet ring cells (SRC), as signs of intracellular mucin formation were seen in all cases, but were generally scattered and low in numbers, although they could be readily identified in some cases. SRCs were seen in more abundance outside of the mucinous area of case 3 (Fig. 2f). The highest number of SRCs was seen in the metachronous omental metastasis of case 2; the primary tumour also had some SRCs, but these did not represent a characteristic feature of the tumour.

Axillary lymph node metastases were observed in five patients; only one showed areas of extracellular mucin production, in keeping with the lobular mixed phenotype; the others demonstrated non-mucinous ILC phenotype. The two local recurrences were evaluated by histology and were devoid of extracellular mucin on review too. Of the two distant metastases occurring in the course of the follow-up, only one was examined by microscopy, and displayed abundant extracellular mucin and high numbers of SRCs (Fig. 1f). The autopsy material of case 2 was not available for review.

All tumours including the ILC-ECMUC areas were negative for E-cadherin and demonstrated cytoplasmic p120 catenin staining. Membranous β-catenin staining was completely absent from six tumours, and was partially present in two (Fig. 3). This involved the mucinous component and ranged from focal partial membranous or complete circumferential staining of a minority of cells in areas of lumen formation (i.e. glandular differentiation) (Fig. 3d) to nearly complete, diffuse, dominantly circumferential but sometimes only partial membranous or lacking (Fig. 3e). Moreover, similar β-catenin partial staining was also observed in the solid component of case 4, but was definitely absent from the classic type non-mucinous ILC of the same case (Fig. 3f). High-molecular weight cytokeratin (HMWCK) staining seen in many lobular carcinomas [27], was obtained in seven cases and was positive in four. For what concerns neuroendocrine differentiation, all tumours were negative for CD56, and all but one tumour were negative for chromogranin A and synaptophysin. All cases showed cytoplasmic positivity for MUC1 and 7/8 cases showed MUC2 positivity as well, which was generally more expressed in the mucinous area. All tumours were ER positive (ranging from 60 to 100%). PR positivity was inferior to 1% in one case, of low percentage (5%) in two cases, and the rest of the tumours displayed 10 to 90% positivity. Immunohistochemistry for HER2 was positive (3+) in one case and was 1+ or 2+ in two cases which were non-amplified with fluorescent in situ hybridization. The Ki-67 index of the tumours ranged between 7 and 40%, and was generally somewhat lower in the mucinous area (Table 2).

The surgical treatment consisted of mastectomy in six cases and breast conserving surgery in two. Four patients were staged with sentinel lymph node biopsy only, and four had axillary lymph node dissection either without or following sentinel lymphadenectomy. Adjuvant radiotherapy was given or for recently diagnosed cases is planned to be given to seven patients. Aromatase inhibitors were/are part of the treatment for four patients, whereas chemotherapy was given to four patients, one received the treatment in the neoadjuvant setting. One patient having a HER2 overexpressing tumour also received trastuzumab for 1 year along with letrozole.

Since three cases were diagnosed in 2015–2016 and their uneventful follow-up of less than 12 months is not meaningful, only the data of the five remaining patients with longer follow-up (median 29 months, range = 15–68 months) are considered here. Three of them experienced recurrences: one local, 68 months after initial diagnosis; one peritoneal 39 months after diagnosis and finally, one local and distant (liver, bones and brain) 15 months after discovery and initial

Table 2 Some clinical and pathological features of the present and previously reported cases of ILC-ECMUC

Case	Age at diagnosis	Pathological size (mm)	Nodal status	Percent of mucinous area (%)	Grade	Surgery	Adjuvant therapy	Recurrence	Follow-up (months)	ER (%)	PR (%)	HER2	b-cat	HMWCK	MUC2	ChrA/Syn	Ki67 (%)
1	69	>24	1/2	30	G2	M + SNB	RT + HT	No	26	100	10	−	−	+	+	−	20
2	65	90	11/13	25	G2	BCS > M	RT + CT + HT	Yes[a]	40	90	5	−	−	+	+	−	30
3	71	46	0/3	30	G2	BCS + SNB	RT + HT + trast	No	29	90	40	3+	−	−	−	−	10
4	62	80	10/23	50	G2	BCS > M + ALND	RT	Yes[b]	68	90	80	NAmp	+ (f-oc)	x	+	−	40
5	45	29	0/2	10	G3	BCS + SNB	CT + RT	No	2	95	95	−	−	−	+	+	40
6	56	22	0/1	70	G2	M + SNB	RT	No	11	100	70	−	+ (f-oc)	+	+	−	20
7	75	30	7/9	5	G2/yG3	M + ALND	NeoCT	Yes[c]	21	80	<1	−	−	−	+	−	20
8	60	50	3/13	50	G2	M + ALND	CT + RT + HT	No	NA	60	5	NAmp	−	+	+	−	7
Rosa [18]	60	90	x	20	x	M	x	x	x	x	x	x	x	x	x	x	x
Yu [19]	65	x	1/ni	x	x	BCS + SNB	x	x	x	100	−	3+	ND	x	−	x	25
Haltas [20]	43	x	1/19	x	x	M + ALND	x	x	x	+	+	−	x	x	x	−	x
Bari [21]	38	35	2/10	x	x	M + ALND	x	x	x	+	+	−	x	x	x	−	x
Gomez Macias [22]	60	9	0/4	x	G1	BCS + SNB	RT + HT	x	x	+	90	−	x	x	x	x	x

− negative, + positive, *ALND* axillary lymph node dissection, *b-cat* beta-catenin, *BCS* breast conserving surgery, *ChrA* chromogranin A, *CT* chemotherapy, *ER* oestrogen receptor, *G* histological grade, *HER2* human epidermal growth factor receptor 2, *HMWCK* high-molecular weight cytokeratin, *HT* hormonal treatment, *foc* focal, *M* mastectomy, *NA* not applicable, *NAmp* non-amplified (cases testing 1+ or 2+ on immunohistochemistry), *neo* neoadjuvant, *ni* no information, *PR* progesterone receptor, *RT* radiotherapy, *SNB* sentinel node biopsy, *Syn* synaptophysin, *trast* trastuzumab, *x* not done/no data

[a] Peritoneal metastasis

[b] Ipsilateral breast recurrence

[c] Local recurrence and distant metastasis to liver, bones and brain

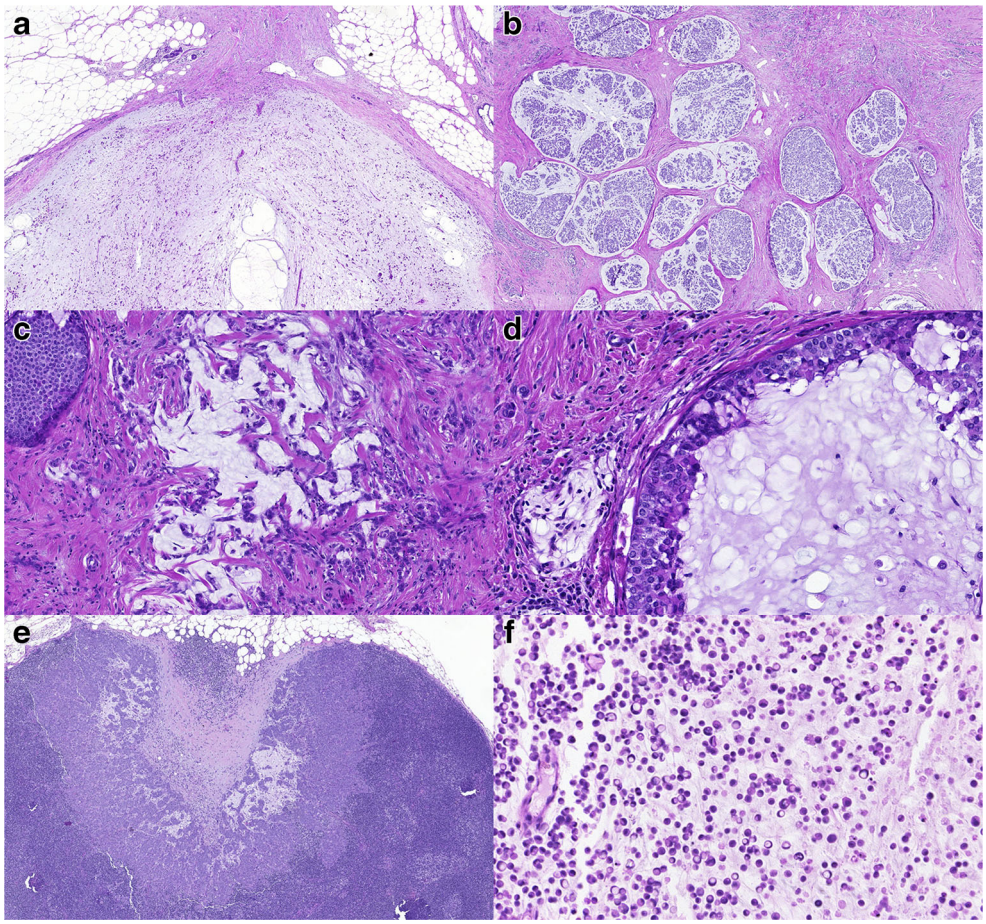

Fig. 1 Mucinous areas in lobular carcinomas with extracellular mucin production and its metastases. Uninodular (**a** HE, ×5, case 3); multinodular (**b** HE, ×5, case 6) and non-circumscribed (**c** HE, ×20, case 1) mucinous areas. Note the lobular neoplasia in the *upper left corner* (**c**). **d** Lobular neoplasia with extracellular mucin production (HE, ×40, case 1). **e** Lymph node metastasis with mucinous component (HE, ×6, case 1); **f** Pure mucinous distant metastasis (HE, ×40, case 2).

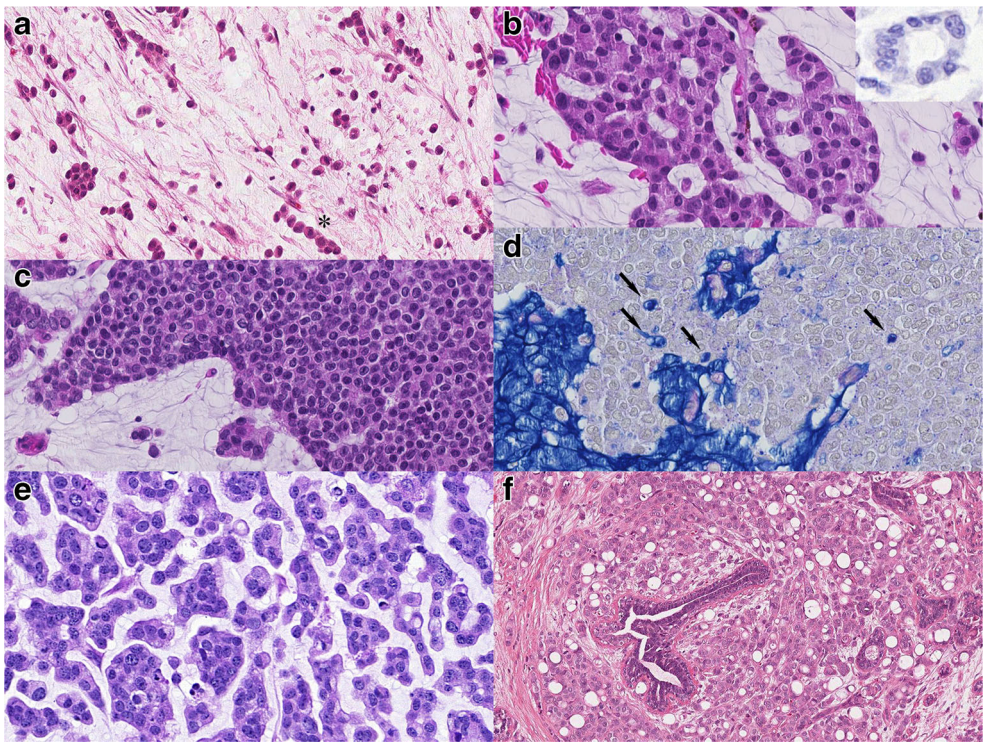

Fig. 2 Morphological patterns of ILC-ECMUC. **a** Paucicellular pattern with discohesive cells sometimes arranged in single-cell lines (*asterisk*) (×20, case 3). **b** Pseudocribriform or pseudoglandular structures with mucin filled lumina; *inset* single tubule-like lumen (HE, ×40, case 4; *inset* beta-catenin, ×72, case 8). **c**, **d** Solid sheet-like clusters of cells reminiscent of lobular carcinoma in situ. Some cells contain intracellular mucin as highlighted by Alcian blue (*arrows*). (HE (**c**) and Alcian blue (**d**), ×40, case 4). **e** Groups of cell floating in pools of mucin, reminiscent of classical mucinous carcinoma; some of the cells contain intracytoplasmic vacuoles (HE, ×72, case 2). **e** Non-mucinous component demonstrating higher number of signet ring cells (HE, ×20, case 3)

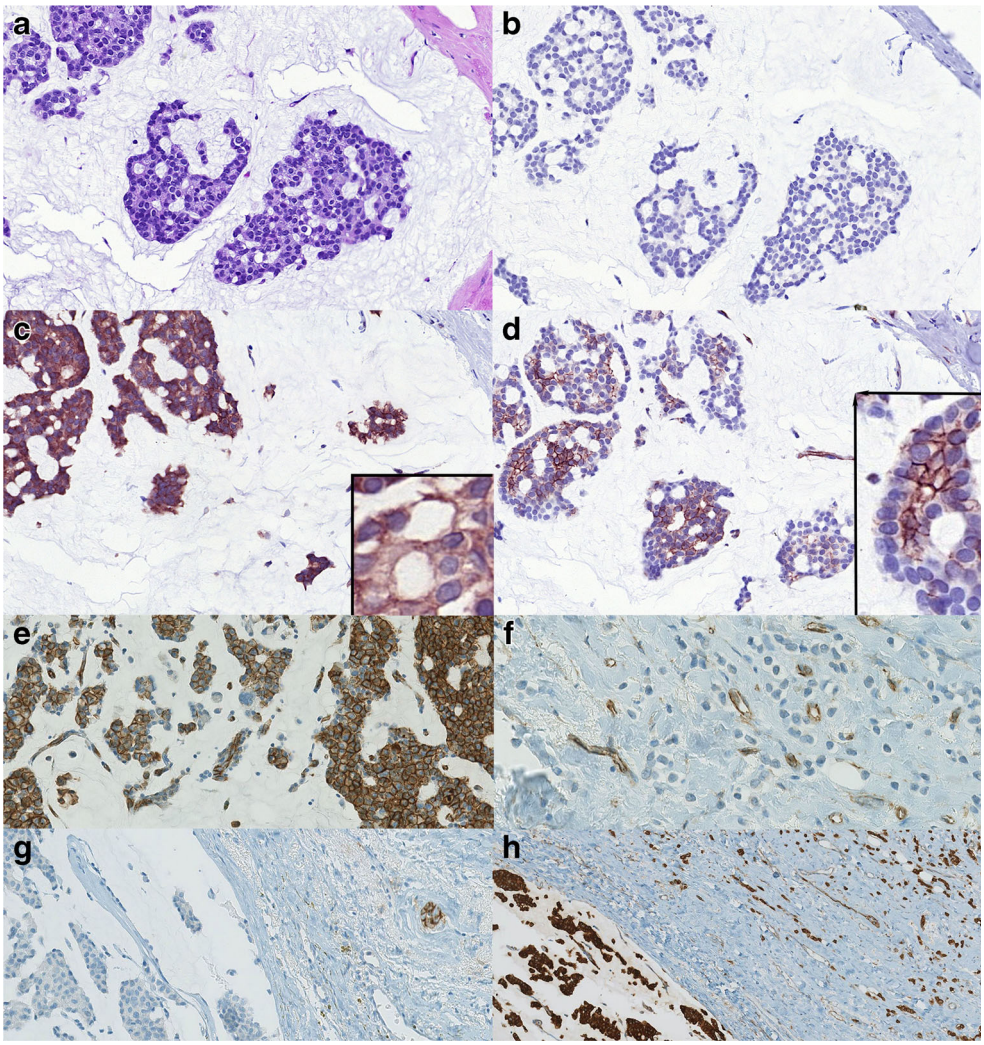

Fig. 3 Two cases with discordant lobular carcinoma immunohistochemistry. **a**–**d** Approximately same area of case 6 with **a** H&E, **b** E-cadherin, **c** p120 catenin and **d** beta-catenin stains (All ×40 originally; *insets* ×70). While E-cadherin is negative in the mucinous area, and p120-catenin shows a cytoplasmic staining in keeping with the diagnosis of lobular carcinoma, beta-catenin is focally and weakly positive in the mucinous area (not in the surrounding classic lobular carcinoma—not shown). **e**–**h** Case 4, beta-catenin more diffusely and strongly but still not completely staining the mucinous part (**e**) and being completely negative in the surrounding classic type (**f**, both ×40). The E-cadherin staining (**g**, ×20) is negative in both components, whereas the p120-catenin (**h**, ×10) staining is cytoplasmic as expected in lobular carcinomas

treatment. The latter two patients died of their disease 40 and 21 months after recognition of the tumours, respectively.

Discussion

The cases described here correspond to the entity previously reported in single-case reports as ILC-ECMUC [19–23]. Although the areas of the tumours demonstrating lakes of extracellular mucin could be classified as mucinous on the basis of the "neoplastic cells floating within extracellular mucins" definition [11], and could also be labelled as mucinous carcinoma with lobular features, the authors all preferred the alternative labelling of these tumours as ILC-ECMUC or ILC with mucinous features, in keeping with earlier single-case reports [19–23]. The rationale for this preference in terminology includes a classical ILC component present around the mucinous area in most cases, the presence of associated LN, a precursor lesion, in most cases, and the fact that cells in the mucinous area, whether clustered, lumen forming or not, also showed alterations of the E-cadherin-membrane complex characteristic of lobular carcinomas. It is recognized that some ILCs may show formation of rare glandular structures, which are E-cadherin negative (Fig. 2b) and this should not refute the diagnosis of an ILC [18], and more complex lumen-like structures (Fig. 2b) were also considered analogously, i.e. not refuting ILC by being E-cadherin negative. In cases of aberrant E-cadherin staining, p120 and β-catenin are recommended as additional IHC stains that may clarify the histological type [18]. Aberrant β-catenin membranous stain in the lack of membranous expression of E-cadherin and p120 has not been studied, but the latter stains are in keeping with lack of integrity of the E-cadherin-membrane complex.

Acknowledging ILC-ECMUC as a distinct variant of ILC, examples demonstrating this phenotype along with classic ILC or other variants (e.g. the solid) could be categorized as ILC mixed type. Reviewing the case reports [19–23] and the present series suggests that these tumours always included smaller or larger areas of non-mucinous ILC, generally of

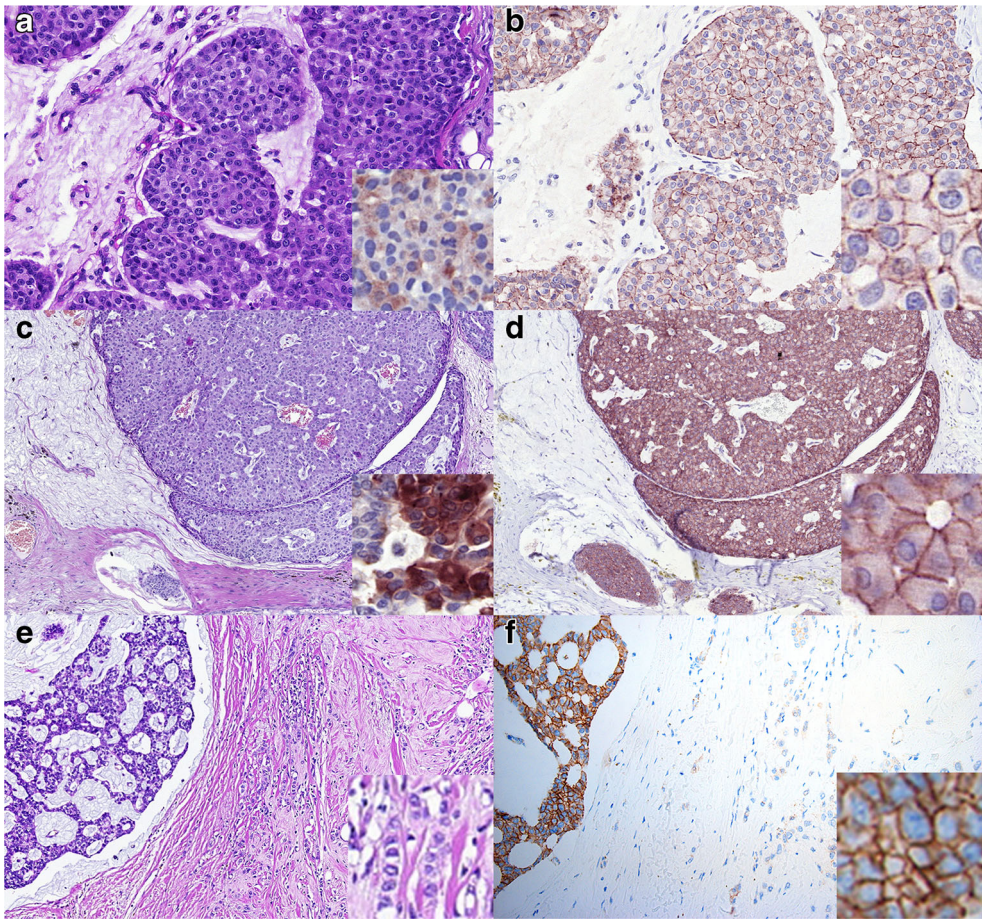

Fig. 4 Differential diagnostic considerations. **a**, **b** Mucinous carcinoma, type B by Capella demonstrating neuroendocrine differentiation and membranous staining with the E-cadherin-membrane complex proteins. (**a** H&E ×40, *Inset* Chromogranin A ×40; **b** E-cadherin ×40, *Inset* ×70). **c**, **d** Solid papillary carcinoma with area of extracellular mucin formation (**c** H&E ×15, *Inset* synaptophysin ×40; **d** p120 ×15, *Inset* ×70). **e**, **f** Composite tumour with mucinous carcinoma (*left*) and lobular carcinoma (*right*) (**e** H&E ×20, *Inset* ×40; **f** β-catenin ×20, *Inset* ×40)

the classical, sometimes additionally of the solid type. Of course, this does not exclude the possibility of a pure ILC-ECMUC. As can be expected in case of mixed type tumours, heterogeneity is also present in their metastases, as exemplified by only 1/5 of node-positive ILC-ECMUCs showing an area of extracellular mucin formation in the lymph nodes. Only two of the three previous case reports give relevant details in respect of nodal involvement [19–22], and only one of the cases showed the features of ILC-ECMUC in nodal metastases [22]. Case 2 of the present series deserves special attention: it had 11/13 lymph nodes involved, and none of these showed mucinous features, whereas the only slide of an omental metachronous distant metastasis developing 39 months after primary surgical treatment showed a pure ILC-ECMUC with relatively high numbers of SRCs (Fig. 1f). The other two cases with local failures showed no extracellular mucin formation in the recurrence.

The low yield of the search suggests that this is a rare morphological entity, although it must also be underdiagnosed, as some earlier cases were primarily diagnosed as (mixed) mucinous (MBC) or ductal (no special type) carcinomas with mucin formation. Owing to the fact that a core needle biopsy may sample the mucinous part only, where the cells are sometimes clustered and lumen forming, a low power examination may yield a "false" diagnosis of MBC. Looking for radiologic correlation in a multidisciplinary setting may help in avoiding such misdiagnoses, as classical MBCs are typically circumscribed, whereas ILCs are not uncommonly seen as architectural distortions or spiculated masses, as some of the present series, or a previous case report [23]. Hitting the non-mucinous area may obviously result in the diagnosis of ILC without extracellular mucin formation, as was the case in two instances of the present series.

Previous reports have all identified SRCs in ILC-ECMUC in varying amounts as an evidence of intracellular mucin accumulation. Both these cells and extracellular mucin showed PAS, mucicarmin and Alcian blue staining when investigated for these stains. Likewise, SRCs were also seen in all cases of the present series, ranging from rare to strikingly evident, although the area with most SRCs in a primary tumour was outside of the mucinous area (Fig. 2f). In keeping with previous reports, the extracellular mucin was positive with Alcian blue in all cases and showed strong or weaker PAS positivity as well, similarly to varying amounts of intracellular mucin. It must be noted that in their description of five SRC carcinomas as variants of ILC, Steinbrecher and Silverberg illustrate a case (number 3 in their series) with extracellular mucin formation without paying much attention to this feature they

describe [3]. Worth mentioning, Gad and Azzopardi had described (intracellular) mucin production of lobular carcinomas a year earlier [28].

The mucin composition of a case of ILC-ECMUC was analysed in details earlier. The case was found to show positivity for MUC1, but was negative for MUC2, MUC4, MUC5AC and MUC6 [20]. Our series demonstrated positivity for the secretory mucin MUC2 in 7/8 cases.

Neuroendocrine differentiation can occur in several breast cancer types including LN [29] and has been often seen associated with (intracellular or extracellular) mucin production [30]. It was present in one case of the present series. Neuroendocrine markers were investigated in only two previous cases reported and were negative [21, 22]. This may be of importance in the differential diagnosis of this tumour subtype, since mucinous carcinoma of type B after Capella often shows neuroendocrine differentiation [31] along with membranous expression of E-cadherin and other members of the E-cadherin-membrane complex (Fig. 4a, b).

Another tumour that may come into the differential diagnosis is solid papillary carcinoma, which also often features neuroendocrine differentiation and can be associated with mucin production and even mucinous carcinoma [32]. However, this tumour also shows a non-lobular phenotype as concerns the E-cadherin-membrane complex (Fig. 4c, d). Sometimes mixed ILC and MBC may also occur, as a combination of the two distinct histological types (Fig. 4e, f). The evaluation of E-cadherin staining should also help in distinguishing these two distinct phenotypes. Due to their mucoid/myxoid stroma, matrix-producing metaplastic carcinomas [33] and polymorphous mammary adenocarcinomas [34] may also enter the differential diagnosis, especially as they may show lacking or altered E-cadherin expression [34, 35], and polymophous carcinoma may also mimic the Indian-filing pattern of classic ILC [34, 36]. However, both these latter tumours are typically ER-negative and have other features distinguishing them from ILC [10, 11]. In the metastatic setting, mucin-producing tumours of other organs come also into consideration, and SRC carcinomas (most often of gastric origin) must also be thought about, since they share features with ILC-ECMUC, including the lack of E-cadherin staining.

The reasoning described at the beginning of the discussion led us and others to classify the morphology seen in the cases of the present series as representing ILC of mixed type, with a component forming extracellular mucin (ILC-ECMUC). As the production of extracellular mucin is rare in lobular carcinomas (which are generally known to produce intracellular mucin), we have included all cases demonstrating this feature into the category of ILC-ECMUC, independently of the proportion of the tumour showing it. We believe that recognising the entity of ILC-ECMUC is important because of its mimic of mucinous carcinoma and other mucin-producing carcinomas which may have completely different diagnostic, therapeutic and prognostic implications. It is impossible to draw conclusions about the natural history of these tumours due to the low number of cases and to the presence of a substantial to dominant non-mucin-forming component in all cases seen to date. Therefore, we must restrict the conclusions to the morphology of these cases which might be misperceived or misdiagnosed as mucinous carcinomas of pure or mixed type both in biopsies of the primary tumour or its metastases. Recognition of this rare but also underdiagnosed morphology/entity and the availability of a cell line model [37] may lead to a better understanding of its formation and biology.

Acknowledgements The authors thank the help of Drs. Susanne Zöch and Sigurd Lax (Landeskrankenhaus Graz Süd-West, Graz, Austria); Dr. Vasiliki Zolota (University of Patras, Greece); Dr. Georgia Kafiri (Hippokrateio Hospital of Athens, Greece); Dr. Paivi Heikkila (University of Helsinki, Finland); Drs. Celia Perdaens and Veerle Delvaux (Jan Portaels Hospital, Vilvvorde, Belgium); and Dr. Janina Kulka (Semmelweis University, Budapest, Hungary) for their help in collecting the cases and or relevant clinical details. This study was partially funded by the National Research, Development and Innovation Office grant GINOP-2.3.2-15-2016-00020.

Author Contributions All authors of the manuscript made substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work; and drafting the work and/or revising it critically for important intellectual content; and final approval of the version submitted for publication and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Other contribution is acknowledged in the Acknowledgements section of the manuscript.

Compliance with ethical standards The authors have consulted the journal policy regarding compliance with ethical standards and state that accepted principles of ethical and professional conduct have been followed. The authors include information regarding sources of funding and potential conflicts of interest (financial or non-financial). Ethical approval and informed consent related information (waiver for this particular study) are summarized in the final paragraph of the "Material and Methods". The study did not include animals, therefore issues relating to animal welfare do not apply.

Funding This study was partially funded by the National Research, Development and Innovation Office grant GINOP-2.3.2-15-2016-00020.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Foote FW Jr, Stewart FW (1941) Lobular carcinoma in situ: a rare form of mammary cancer. Am J Pathol 17:491–496
2. Fechner RE (1975) Histologic variants of infiltrating lobular carcinoma of the breast. Hum Pathol 6:373–378
3. Steinbrecher JS, Silverberg SG (1976) Signet-ring cell carcinoma of the breast. The mucinous variant of infiltrating lobular carcinoma? Cancer 37:828–840

4. Fisher ER, Gregorio RM, Redmond C, Fisher B (1977) Tubulolobular invasive breast cancer: a variant of lobular invasive cancer. Hum Pathol 8:679–683
5. Martinez V, Azzopardi JG (1979) Invasive lobular carcinoma of the breast: incidence and variants. Histopathology 3:467–488
6. Page DL, Anderson TJ, eds (1987) Diagnostic histopathology of the breast. Churchill Livingstone, Edinburgh
7. Weidner N, Semple JP (1992) Pleomorphic variant of invasive lobular carcinoma of the breast. Hum Pathol 23:1167–1171
8. Eusebi V, Magalhaes F, Azzopardi JG (1992) Pleomorphic lobular carcinoma of the breast: an aggressive tumor showing apocrine differentiation. Hum Pathol 23:655–662
9. Amendoeira I, Apostolikas N, Bellocq JP et al (2006) Quality assurance guidelines for pathology. In: Perry N, Broeders M, de Wolf C, Törnberg S, Holland R, von Karsa L (eds) European guidelines for quality assurance in breast cancer screening and diagnosis, 4th edn. European Comission, Luxemburg, pp 219–311
10. Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ (eds) (2012) WHO classification of tumours of the breast. International Agency for Research on Cancer, Lyon
11. Tavassoli FA, Eusebi V (2009) Tumors of the mammary gland. Armed Forces Institute of Pathology Atlas of tumor pathology, Series 4. American Registry of Pathology, Washington, DC
12. Cserni G (1999) Reproducibility of a diagnosis of invasive lobular carcinoma. J Surg Oncol 70:217–221
13. Acs G, Lawton TJ, Rebbeck TR, LiVolsi VA, Zhang PJ (2001) Differential expression of E-cadherin in lobular and ductal neoplasms of the breast and its biologic and diagnostic implications. Am J Clin Pathol 115:85–98
14. Kovács A, Dhillon J, Walker RA (2003) Expression of P-cadherin, but not E-cadherin or N-cadherin, relates to pathological and functional differentiation of breast carcinomas. Mol Pathol 56:318–322
15. Rakha EA, Abd El Rehim D, Pinder SE, Lewis SA, Ellis IO (2005) E-cadherin expression in invasive non-lobular carcinoma of the breast and its prognostic significance. Histopathology 46:685–693
16. Da Silva L, Parry S, Reid L, Keith P, Waddell N, Kossai M, Clarke C, Lakhani SR, Simpson PT (2008) Aberrant expression of E-cadherin in lobular carcinomas of the breast. Am J Surg Pathol 32:773–783
17. Rakha EA, Teoh TK, Lee AH, Nolan CC, Ellis IO, Green AR (2013) Further evidence that E-cadherin is not a tumour suppressor gene in invasive ductal carcinoma of the breast: an immunohistochemical study. Histopathology 62:695–701
18. Canas-Marques R, Schnitt SJ (2016) E-cadherin immunohistochemistry in breast pathology: uses and pitfalls. Histopathology 68:57–69
19. Rosa M, Mohammadi A, Masood S (2009) Lobular carcinoma of the breast with extracellular mucin: new variant of mucin-producing carcinomas? Pathol Int 59:405–409
20. Yu J, Bhargava R, Dabbs DJ (2010) Invasive lobular carcinoma with extracellular mucin production and HER-2 overexpression: a case report and further case studies. Diagn Pathol 5:36
21. Haltas H, Bayrak R, Yenidunya S, Kosehan D, Sen M, Akin K (2012) Invasive lobular carcinoma with extracellular mucin as a distinct variant of lobular carcinoma: a case report. Diagn Pathol 7:91
22. Bari VB, Bholay SU, Sane KC (2015) Invasive lobular carcinoma of the breast with extracellular mucin. A new rare variant J Clin Diagn Res 9:ED05–ED06
23. Gomez Macias GS, Pérez Saucedo JE, Cardona Huerta S, Garza Montemayor M, Villarreal Garza C, Garcia Hernandez I (2016) Invasive lobular carcinoma of the breast with extracellular mucin: a case report. Int J Surg Case Rep 25:33–36
24. Hammond ME, Hayes DF, Dowsett M et al (2010) American Society of ClinicalOncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Arch Pathol Lab Med 134:e48–e72
25. Wolff AC, Hammond ME, Hicks DG et al (2014) Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. Arch Pathol Lab Med 138:241–256
26. Tot T (2007) Clinical relevance of the distribution of the lesions in 500 consecutive breast cancer cases documented in large-format histologic sections. Cancer 110:2551–2560
27. Bratthauer GL, Moinfar F, Stamatakos MD et al (2002) Combined E-cadherin and high molecular weight cytokeratin immunoprofile differentiates lobular, ductal, and hybrid mammary intraepithelial neoplasias. Hum Pathol 33:620–627
28. Gad A, Azzopardi JG (1975) Lobular carcinoma of the breast: a special variant of mucin-secreting carcinoma. J Clin Pathol 28:711–716
29. Eusebi V, Azzopardi JG (1980) Lobular endocrine neoplasia in fibroadenoma of the breast. Histopathology 4:413–428
30. Sapino A, Righi L, Cassoni P, Papotti M, Pietribiasi F, Bussolati G (2000) Expression of the neuroendocrine phenotype in carcinomas of the breast. Semin Diagn Pathol 17:127–137
31. Capella C, Eusebi V, Mann B, Azzopardi JG (1980) Endocrine differentiation in mucoid carcinoma of the breast. Histopathology 4:613–630
32. Tan PH, Schnitt SJ, van de Vijver MJ, Ellis IO, Lakhani SR (2015) Papillary and neuroendocrine breast lesions: the WHO stance. Histopathology 66:761–770
33. Wargotz ES, Norris HJ (1989) Metaplastic carcinomas of the breast. I. Matrix-producing carcinoma. Hum Pathol 20:628–635
34. Asioli S, Marucci G, Ficarra G, Stephens M, Foschini MP, Ellis IO, Eusebi V (2006) Polymorphous adenocarcinoma of the breast. Report of three cases. Virchows Arch 448:29–34
35. Petersson F, Putti TC (2010) Multifocal matrix-producing mammary carcinoma mimicking lobular carcinoma on core biopsy - a case with long survival reported. Pathol Res Pract 206:527–531
36. Nicol KK, Iskandar SS (2000) Lobular carcinoma of the breast metastatic to the oral cavity mimicking polymorphous low-grade adenocarcinoma of the minor salivary glands. Arch Pathol Lab Med 124:157–159
37. Jambal P, Badtke MM, Harrell JC, Borges VF, Post MD, Sollender GE, Spillman MA, Horwitz KB, Jacobsen BM (2013) Estrogen switches pure mucinous breast cancer to invasive lobular carcinoma with mucinous features. Breast Cancer Res Treat 137:431–448