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ORIGINAL ARTICLE

Elastic stains in the evaluation of DCIS with comedo necrosis in breast cancers

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Abstract As concerns the microscopic morphology of ductal carcinoma in situ (DCIS), neoplastic cells are surrounded by both a myoepithelial cell layer and a basement membrane as expected from the outer structure of ducts and lobules. However, in some cases, it is impossible to state whether the structures involved by the disease are ducts or lobules. Altogether 1220 anatomic structures involved by DCIS displaying comedo necrosis from 27 slides of 21 patients (seen on both haematoxylin and eosin-stained and orcein-stained slides) were identified as representing ducts, likely ducts, unclassifiable structures, likely acini or acini on the basis of their distribution and resemblance to normal anatomic structures. All structures were then rated as having a circumferential elastic layer (as normal ducts), a partial elastic layer around more or less than half of the periphery or having no peripheral elastic layer at all (as normal acini). Structures classified as ducts or likely ducts were likely to have an elastic coating, whereas acini and likely acini had no such coating. Unclassifiable structures were generally devoid of an elastic layer. Structures (and cases) that were likely to represent neoductgenesis as proposed by Zhou et al. (Int J Breast Cancer 2014;2014:581706) were generally unclassifiable and devoid of outer elastic layer. Many duct-like structures in DCIS with comedo necrosis are devoid of elastic layer typical of normal ducts, suggesting that these structures are abnormal despite conservation of the myoepithelium and the basement membrane.

Keywords Breast cancer · Comedo necrosis · Ductal carcinoma in situ · Elastic stain · Neoductgenesis

Introduction

Breast parenchyma is organised into anatomic units corresponding to mammary lobes. A lobe can be defined as the complex of a lactiferous duct branching into smaller and smaller ducts with terminal ductules and the lobule forming acini belonging to these ductules at the end, i.e. each lactiferous duct defines a different lobe, of which 15 to 25 make up the breast parenchyma [1]. An easy to imagine visual analogy of the lobar organisation would be that of a tree, where the lactiferous duct would correspond to the trunk, the ducts to branches and the lobules to compound leaves. The outer myoepithelial layer and the basement membrane around the ducts would represent the bark of the tree. Acini of the lobules and ducts are easy to distinguish from each other. However, when their lumen enlarges and the diameter becomes larger, ectatic ducts and cystically dilated acini are not always easy to separate. The maintained lobular architecture, multiplicity of the lumens and the common presence of apocrine epithelium helps to identify dilated lumina as cysts. In contrast to cysts, ectatic ducts are generally single or separated from each other by normal breast adipose or fibrous tissue more in keeping with interlobular stroma than with intralobular stroma. Although both the ducts and lobules feature an outer myoepithelial cell layer and a basement membrane, there is usually also an elastic layer around the ducts, which is missing around the acini (Fig. 1). This phenomenon can also help in the distinction between ectatic ducts or cystic acini, although

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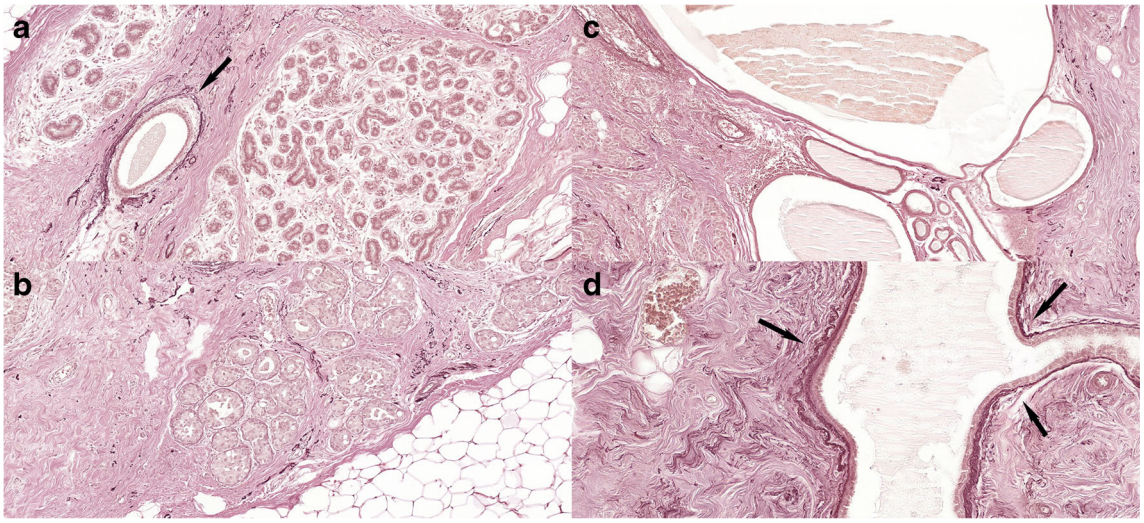

Fig. 1 Elastic fibres around normal and dilated anatomic structures. **a** A duct with elastic coating (arrow) and acini without an outer elastic layer arranged into lobules. **b** Lobular cancerisation. DCIS extending into a lobule without altering the lack of elastic fibres around lobule forming acini. **c** Closely packed cysts devoid of elastic fibres. **d** Ectatic duct with maintained elastic coating (arrows). (Orcein, ×10, ×10, ×8, ×15)

the amount of elastic fibres around dilated ducts is dependent on the amount initially present and any damage by inflammatory processes (Fig. 1) [2].

When ductal carcinoma in situ (DCIS) develops in the ductal tree or its end, the terminal ductulolobular unit (TDLU), there is often a dilatation of the normal anatomic structures. When the anatomy of the breast is maintained, DCIS can easily be identified as involving the ducts and sometimes the lobules. The latter phenomenon has been called cancerisation of lobules or lobular cancerisation [3, 4] (Fig. 1b). The analogy of the tree also applies to this presentation.

Some forms of DCIS do not follow this regular growth and intraluminal spread. Instead, there are plenty of more or less dilated lumens dispersed with a relatively uniform and higher than normal density. Although some believe that this pattern is also due to abnormal extent of dilatation of preexisting structures (i.e. ducts and lobules) like overinflated balloons touching each other, there is apparently no obviously discernible lobular architecture maintained. The concept of neoductgenesis has been introduced to explain the morphology of these cancers [5, 6]. This concept suggests that new ducts are formed from the preexisting ones and grow into the stroma. Keeping the analogy of the tree, this would correspond to new lateral branches starting to grow from the trunk or other larger branches, keeping the characteristic feature of branches, i.e. having their bark. Indeed, the structures identified under the microscope in such cases have a myoepithelial layer and a basement membrane and are easy to interpret as DCIS, although myoepithelial phenotype and myoepithelial marker expression may be altered [7, 8]. Newly formed ducts 'invade' the stroma in a pushing manner without losing their myoepithelial and basement membrane layers which are the diagnostic hallmark of in situ carcinomas and the clue used to exclude the presence of invasion in breast pathology. This controversy is certainly the cause why the concept of neoductgenesis is not widely accepted, despite having more and more advocates. Neoductgenesis could explain the aggressive behaviour of tumours with this phenomenon having minor areas of classical invasive carcinoma of no special type (interpreted as ductal carcinomas with extensive intraductal component). Such tumours have a larger whole tumour size (including the DCIS morphology) than invasive tumour size (including only the smaller classical invasive carcinoma), and when stratified according to other prognosticators, their survival curves match those of no special type invasive tumours of which the size corresponds to the whole tumour size of tumours with neoductgenesis rather than their classical invasive tumour size [5, 6]. The phenomenon could also be an explanation for the anecdotal cases where no classical invasive tumour is identified near DCIS with features of neoductgenesis, but the patient dies of breast cancer [6]. Local recurrence rates in cases diagnosed as representing pure DCIS are also higher in neoplasms with proposed neoductgenesis, and this is also related to their greater extension and diffuse growth pattern [9, 10] but also reflects a worse overall prognosis.

Neoductgenesis is not easy to define. It has been associated with casting (linear branching) type calcifications on the mammogram, and tumours with this manifestation have been reported to have poor outcome in several series [5, 11–14] with some contradicting results in others [15–17]. Tenascin expression has been demonstrated around the newly formed ducts [5, 6]. Morphologic criteria have also been proposed on the basis of duct concentration, periductal fibrosis and lymphocytic infiltration [18] and may help to identify cases with this phenomenon even on histological slides.

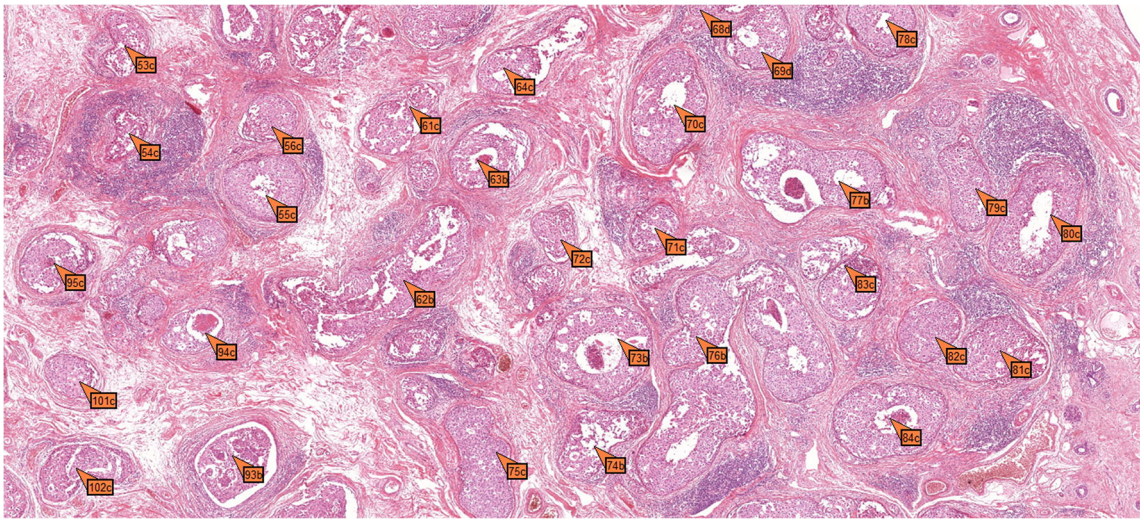

Fig. 2 Annotated structures. Structures that could be identified on both HE and ORC slides were numbered and classified as ducts (**a**), likely ducts (**b**), unclassifiable structures (**c**), likely acini (**d**) or acini (**e**)

In the present study, we analysed lumen forming structures of the DCIS component of a few tumours (some of which were believed to represent DCIS with neoductgenesis) with orcein staining with the aim to see how ducts and acini maintain their staining when involved by DCIS and to see how ducts believed to be newly formed behave with this stain.

Material and methods

Selected histological slides of patients with areas of DCIS (mostly associated with areas of invasive cancer) demonstrating comedo necrosis and central, amorphous microcalcification on histology slides were retrospectively analysed. The study cases were from the Pathology Departments of the University of Szeged (collected from a consecutive series of patients with radiologic evidence of casting (linear branching) type calcification) and the Bács-Kiskun County Teaching Hospital (randomly selected cases with comedo necrosis and central amorphous calcification in the slides; these were from a previous pilot study looking at the elastic stains).The clinicopathological information needed was obtained from the radiological and histopathological reports.

Inclusion criteria were the following: casting (linear branching) type calcification described by the radiological report or the histopathology report, high or intermediate grade DCIS [19] with or without invasive breast carcinoma, resected tumour specimens and availability of slides and paraffin blocks. For control, two slides with normal breast tissue and architecture (derived from non-tumorous random samples of resections performed for breast cancer) were included in the study.

Haematoxylin and eosin (HE) staining was employed for the identification of anatomic structures involved by the in situ neoplastic cells as ducts or acini, whereas orcein staining alone or combined with haematoxylin or HE (ORC) was used for the evaluation of elastic fibres around the units previously classified on the basis of the HE stain. One or two slides per case were selected and digitised with a 3DHistec Pannoramic 250 Flash III scanner.

In the first step, with the aid of the freely downloadable Pannoramic Viewer software (3DHistec, Budapest, Hungary), the structures were numbered in parallel on both the HE and the ORC digital slides (Fig. 2).

Each numbered structure that could be identified on both HE and ORC stained slides was then classified into one of the following categories: definitely representing a duct, likely to be a duct, unclassifiable, likely to be an acinus, and definitely representing an acinus (Fig. 2). For this classification, the resemblance to the normal microanatomical structures was considered, i.e. clustered arrangement into or resemblance to lobular structures pointing to acinar structures and single structures pointing to a ductal nature.

The elastic fibres around the numbered and classified structures were graded as concentric presence (score 3), dominant presence (continuous or discontinuous elastic fibres around more than half of the perimeter of the structure; score 2), dominant absence (focal, elastic fibres around less than half of the perimeter of the structure; score 1) and absence of elastic fibres around the structure (score 0) (Fig. 3).

The neoductgenesis score described by Zhou et al. [18] was applied for the identification of tumours demonstrating neoductgenesis. (The cases were selected before this description was read). This scoring system takes into account the concentration of duct-like structures and loss of normal ductal-lobular architecture, the lymphocytic

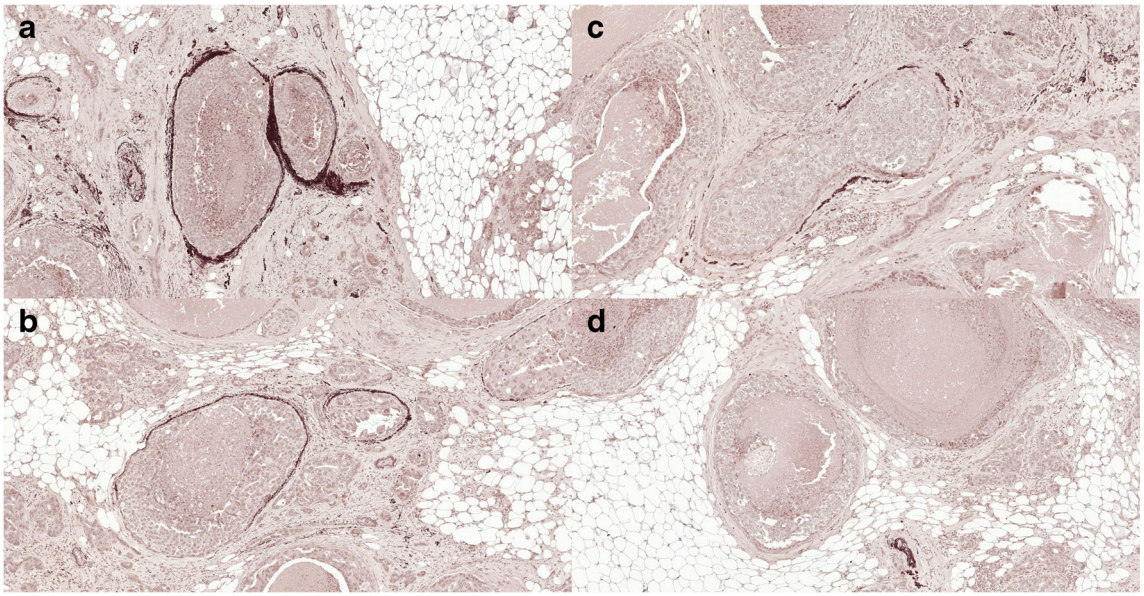

Fig. 3 Scoring of the elastic layer around anatomical structures. **a** Concentric presence (score 3). **b** Dominant presence (continuous or discontinuous elastic fibres around more than half of the perimeter of the structure; score 2). **c** Dominant absence (focal, elastic fibres around less than half of the perimeter of the structure; score 1). **d** Complete or nearly complete absence of elastic fibres around the structure (score 0)

infiltration and periductal fibrosis (Table 1). The original description of the score also includes a visual scale for each score component, and this was used to label the cases. However, we also applied the score to individual structures. For this, we used the first component of the combined score from the entire slide, but the two remaining score components were derived from the lymphocytic infiltration and fibrosis around the given structure.

Table 1 Neoductgenesis score [18]

Variable	Score
Concentration of duct-like structures and loss of normal ductal-lobular architecture	
Absent	0
Focal	1
General	2
Periductal lymphocytic infiltration	
Absent	0
Mild	1
Pronounced	2
Periductal fibrosis	
Absent	0
Mild	1
Pronounced	2
Neoductgenesis	Score
Present	5–6
Absent	0–4

The statistics included the Mann-Whitney and the chi-square tests. All statistical tests were two-sided, and $p < 0.05$ values were considered statistically significant.

The institutional ethical committee of the Bács-Kiskun County Teaching Hospital was consulted and raised no concerns about this non-interventional retrospective study.

Results

Twenty-seven slides from 21 female patients were investigated in this retrospective study (median 1 slide/case). The mean age of the patients was 63.7 (range 46–86). The basic clinicopathological parameters are displayed in Table 2. Two thirds of the patients had high grade DCIS with invasive carcinoma of no special type. The mean ± S.D. invasive tumour size and tumour extension (including DCIS as well) were 13.9 ± 17.7 mm (range = 0–65 mm) and 44.4 ± 30.7 mm (range = 12–65 mm), respectively. The invasive component showed almost exclusively high nuclear grade. The in situ component was of high grade in all but one case. Oestrogen receptor and progesterone receptor positivity were observed in 13 cases and 11 cases, respectively. Intense, concentric membranous positivity of human epidermal growth factor receptor-2 (HER-2) was seen in 13 cases (61.9%). Centrally calcified comedo type necrosis was present in all cases, and casting (linear branching) type calcification on the mammogram was documented in 17 cases; two cases had mammography elsewhere and no data on the type of calcifications were available, and two cases had

Table 2 Basic clinicopathological information in the cases studied

Specimen	
Mastectomy	9 (42.8%)
Breast-conserving surgery	12 (57.2%)
Histological diagnosis	
Pure DCIS	9 (42.8%)
DCIS with invasive carcinoma	12 (57.2%)
Oestrogen receptor	
Positive	13 (61.9%)
Negative	8 (38.1%)
Progesterone receptor	
Positive	11 (52.3%)
Negative	10 (47.7%)
HER-2	
3+	11 (52.3%)
2+	1 (4.8%)
1+	1 (4.8%)
Negative	8 (38.1%)

DCIS ductal carcinoma in situ, *HER-2* human epidermal growth factor receptor-2

granular rather than casting type calcification described in the radiological report.

Altogether 1220 structures were classified as ducts or acini with different levels of certitude, as described in the 'Material and methods'. The majority (nearly two thirds) were unclassifiable, and between 7.4 and 13.6% belonged to one of the four remaining categories each. The relation of these classified structures to peristructural elastic fibres is shown in Table 3. The concentric presence of elastic fibres is more frequent around structures identified as ducts or likely ducts but was also seen around a minority of unclassifiable structures. At the opposite end of the spectrum, acini lack elastic fibres around, similarly to likely acini. Most unclassifiable structures are also completely or partially (dominant absence pattern) devoid of peristructural elastic fibres (Fig. 4).

Ducts (n = 33) and acini (n = 150) of control breast slides were also analysed for the presence of elastic coating around these normal structures. The Mann-Whitney test revealed significant difference between ducts and acini: a concentric elastic fibre layer surrounds almost all normal ducts, but the acini do not have such an elastic coating ($p < 0.001$).

Neoductgenesis was identified in six (25.5%) cases by using the neoductgenesis score suggested by Zhou and coworkers. After the application of this scoring system to all numbered structures, we found that in neoductgenesis positive tumours, 27.3% of the numbered units were suggested as neoducts and there was hardly any potential neoducts (0.75%) in neoductgenesis negative tumours.

We examined the means of the scores of numbered individual structures per case, as well. In neoductgenesis positive tumours (n = 6), the average of the neoductgenesis-related scores was higher (mean ± S.D. = 3.87 ± 0.35, range = 3.49–4.39) than in neoductgenesis negative cases (mean ± S.D. = 1.97 ± 1.11, range = 0–3.1).

Two third of these neoducts identified by the per structure scoring system are irregular and uncertainly classifiable structures (Table 4) and almost 80% of them are not or dominantly not surrounded by elastic fibres. The chi-square test revealed a significant difference between the normal structures and the individual score based neoducts: the latter generally do not have an elastic layer around ($p < 0.001$).

In neoductgenesis positive and negative tumours, the presence of elastic fibres was evaluated by scoring of each structure on orcein-stained slides as described in the 'Material and methods'. Lower scores were found in the neoductgenesis positive tumours (mean ± S.D. = 0.57 ± 0.32, range = 0.28–0.91) than in neoductgenesis negative neoplasms (mean ± S.D. = 0.89 ± 0.54, range = 0.1–2.1).

Table 3 The proportions of elastic fibres around the evaluated structures

Duct vs. acinus	Elastic fibres								All (n)	All (%)
	Absence		Dominant absence		Dominant presence		Concentric presence			
	n	%	n	%	n	%	n	%		
Duct	5	0.4	9	0.7	24	1.9	53	4.4	91	7.4
Likely duct	21	1.3	34	2.9	25	2	33	2.7	113	8.9
Unclassifiable	559	45.9	142	11.7	36	3.1	25	2	762	62.7
Likely acinus	88	7.3	1	0.07	1	0.07	0	0	90	7.44
Acinus	162	13.4	2	0.16	0	0	0	0	164	13.56
All	835	68.3	188	15.53	86	7.07	111	9.1	1220	100

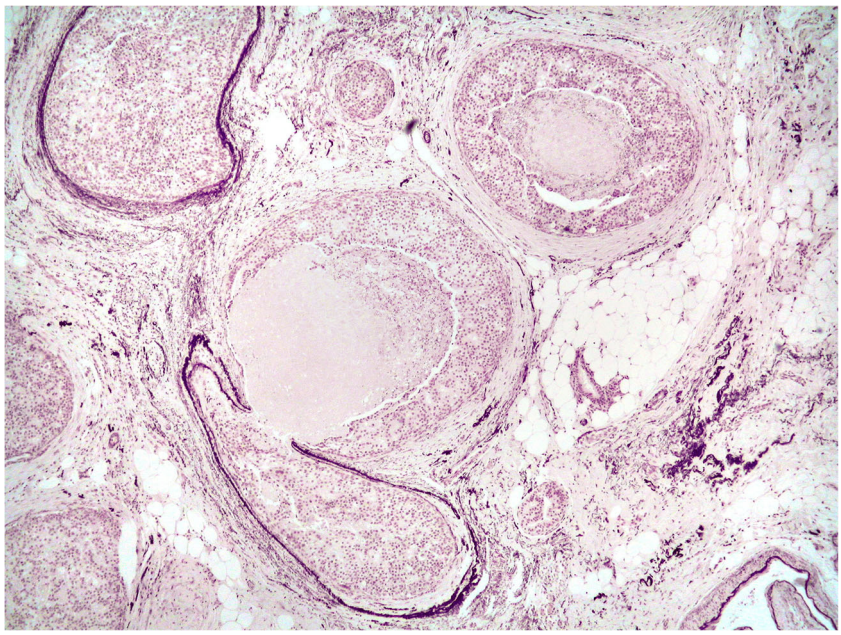

Fig. 4 Elastic fibres around ducts involved by DCIS, and their absence around similar structures, that could represent neoducts but do not reach an individual score for the qualification as neoduct in a neoductgenesis negative case on the basis of the overall score (Orcein, ×4). Note the branching of the elastic fibre-negative structure from the elastic fibre-positive duct

Discussion

Our study of a large number of breast microanatomic structures suggests that most of the densely packed structures involved by morphological DCIS with comedo necrosis cannot be classified on the basis of the HE look as ducts or acini. The study of the control cases reinforced the known microanatomy of ducts and lobules, of which only the previous have an elastic coating. The study also demonstrated that DCIS involved structures that could be classified as ducts or acini with some certainty displayed the elastic coating expected on the basis of the normal anatomic structures. On the other hand, most structures that could not be reliably classified as ducts or acini, in parallel with most structures classified as representing neoducts on the basis of the proposed scoring system [18] adapted to the individual structures, were devoid of an elastic coating. Although this could be used as a support to the acinar origin of these structures, it is more realistic to suggest that together with previously described stromal periductal tenascin-C accumulation [5, 6], this could be another difference between preexisting and newly formed ducts. It is not known whether the proposed new ducts are devoid of periductal elastic fibres from the beginning or these fibres are lost as a consequence of periductal inflammation and/or fibrosis.

Myoepithelial cells have been reported to have an altered phenotype in some DCIS cases, characterised by the loss of one or more myoepithelial markers labelling the myoepithelium around normal anatomic mammary structures. The phenomenon occurs more frequently in high grade DCIS. None of the present cases were of low grade, and all but one case were of high grade. Although we did not study the expression of myoepithelial markers, we detected another

Table 4 The morphological characteristics of potential neoducts ($n = 218$)

Neoduct	Elastic fibres								All (n)	All (%)
	Absence		Dominant abscense		Dominant presence		Concentric presence			
	n	%	n	%	n	%	n	%		
Duct	3	1.4	5	2.4	5	2.4	15	6.9	28	13.1
Likely duct	5	2.4	13	6.2	9	4.2	5	2.4	32	15.2
Unclassified	109	50	20	9.3	5	2.4	5	2.4	139	64.1
Likely acinus	8	3.7	1	0.6	0	0	0	0	9	4.3
Acinus	7	3.3	0	0	0	0	0	0	7	3.3
All	132	60.8	39	18.5	19	9	28	11.7	218	100

abnormality of high grade DCIS cases with comedo necrosis, namely the lack of elastic fibres around the ducts. This was not only present in cases which displayed a neoductgenesis score above 4 but also in cases with lower scores.

Neoductgenesis is a concept to explain the morphology of certain breast cancers that show neoplastic cells in densely packed anatomic structures seemingly corresponding to either normal ducts or acini on the basis of the presence of a basement membrane and a myoepithelial layer at the outer surface but not corresponding to any of these preexisting structures when looking at the lack of the tree-like distribution of normal anatomic structures described in the 'Introduction'. The theory suggests that some breast carcinomas are characterised by the outgrowth of new branches, i.e. new ducts, neoducts from the ductal tree, representing a pushing type infiltration. Densely packed, tumour involved, myoepithelium coated structures could theoretically represent engulfed preexisting acini (and ducts), but when one looks at the phenomenon of lobular cancerization, where this neoplastic inflation happens, or at ductectasia or cystic changes where dilation of preexisting lumina generally occurs without intraluminal epithelial proliferation, it is clear that these processes maintaining the tree-like structure are different from cancers with presumed neoductgenesis. Three-dimensional analysis of such cases further reinforces the lack of normal distribution of ducts and acini in such tumours [6]. This 'disturbed arborisation' [9, 10] could be the result of a disturbed alveolar switch [20], a hormonally driven physiologic mechanism responsible for the development of acini from ductal epithelium. Our report indicates that the proposed neoducts are also devoid of elastic coating, resembling acini in this respect.

There were more structures that could not be reliably classified as ducts or acini than structures corresponding to neoducts on the basis of the neoduct scoring applied to the individual structures. These unclassifiable structures were generally devoid of elastic fibres. Cases that could not be classified as representing neoductgenesis on the basis of the overall score also contained such elastic fibreless unclassifiable structures. It could happen that more of these comedo necrotic structures correspond to neoducts than those identified as neoducts on the basis of the individually (per structure) applied score. Of the two other possible explanations, namely that the unclassifiable structures correspond to acini (originally lacking elastic fibres) or ducts losing their elastic coating, the first seems unlikely due to the diffuse and non-lobular distribution of these structures (this is why they could not be classified even as likely acini), whereas the second is also questionable on the basis of the distribution (there are too many of them clustered close to each other, not allowing their classification even as likely ducts).

Our study therefore identifies possible neoducts as structures devoid of elastic periductal fibres and most of the structures that could not be classified as (likely) ducts or (likely) acini on the basis of distribution patterns as similarly devoid of elastic fibres and possibly also as neoducts. The use of elastic stains may probably be an aid in the study of neoductgenesis.

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Author contribution All authors of the manuscript made substantial contributions to the conception or design of the work; the acquisition, analysis, or interpretation of data for the work; drafting the work and/or revising it critically for important intellectual content; 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.

More specifically, author contribution is listed as follows:

TZ: Case selection, application and refinement of the methods, analysis of data, preparation of the manuscript, finalisation and approval of the manuscript

GC: Concept, development of the methods, case selection, supervision, preparation of the manuscript, finalisation and approval of the manuscript

Compliance with ethical standards

Ethics approval and consent to participate 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 (see Acknowledgment) and potential conflicts of interest (financial or non-financial) (next section). The Institutional Ethical Committee of the Bács-Kiskun County Teaching Hospital was consulted, and no approval was deemed necessary for this retrospective analysis of histological slides requiring no patient identity-related information. An informed consent was not considered possible owing to the anonymity of the tissue sections used for evaluation. The study did not include animals; therefore, issues relating to animal welfare do not apply.

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

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