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ORIGINAL ARTICLE

Morphological intratumor heterogeneity in ductal carcinoma in situ of the breast

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Abstract

Ductal carcinoma in situ (DCIS) of the breast is a heterogeneous disease in terms of morphological characteristics, protein expression profiles, genetic abnormalities, and potential for progression. Molecular heterogeneity has been extensively studied in DCIS. Yet morphological heterogeneity remains relatively undefined. This study investigated morphological intratumor heterogeneity in a series of 51 large DCIS. Nuclear atypia, DCIS architecture, necrosis, calcifications, stromal architecture, and stromal inflammation were assessed in one biopsy slide and three representative slides from each corresponding resection. For each histopathological feature, a histo-score was determined per slide and compared between the biopsy and the resection, as well as within a single resection. Statistical analysis comprised of Friedman tests, post hoc Wilcoxon tests with Bonferroni corrections, Mann-Whitney *U* tests, and chi-square tests. Despite substantial morphological heterogeneity in around 50% of DCIS, the histopathological assessment of the biopsy did not statistically significantly differ from the resection. Morphological heterogeneity was not significantly associated with patient age, DCIS size, or type of surgery, except for a weak association between heterogeneous stromal inflammation and smaller DCIS size. At the group level, the degree of heterogeneity did not significantly affect the representativity of a biopsy. At the individual patient level, however, the presence of necrosis, intraductal calcifications, myxoid stromal changes, and high-grade nuclear atypia was underestimated in a minority of DCIS patients. This study confirms the presence of morphological heterogeneity in DCIS for all six evaluated histopathological features. This should be kept in mind when taking biopsy-based treatment decisions for DCIS patients.

Reports of preliminary data:
- The data reported in this manuscript have partially been presented during the virtual ESMO Breast Cancer meeting (May 2020), and the corresponding abstract has been published in *Annals of Oncology*.
- The data of this original article were presented as a poster during the virtual 32nd ESP Conference (December 2020).

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Introduction

The systematically organized screening mammography has substantially increased the incidence of ductal carcinoma in situ (DCIS), nowadays representing 20–25% of all newly screen-detected breast cancers [1, 2]. DCIS is characterized by a cohesive clonal proliferation of epithelial cells within mammary ducts and terminal ductal-lobular units (TDLUs), without breaching the myoepithelial and basement membrane layers [3, 4]. DCIS is considered as a nonobligate precursor of invasive breast cancer. It is estimated that a substantial number of DCIS are indolent and will never progress to invasive cancer. Some DCIS even present with morphological signs of regression [5]. As it is currently impossible to distinguish indolent from aggressive DCIS, there is considerable uncertainty about the optimal personalized treatment, resulting in a "one size fits all" therapeutic approach [6].

DCIS does not represent a single entity, as it is a heterogeneous disease. Lesions differ with regard to their clinical presentation, morphological characteristics, protein expression profiles, genetic abnormalities, and potential for progression [4, 7]. Although intratumor heterogeneity in DCIS is generally accepted as an established fact, only a few studies have investigated the degree of morphological heterogeneity and its potential impact on the representativity of DCIS biopsies [8–10]. The various classification systems for DCIS grading are also characterized by heterogeneity, as some classifications take into account DCIS architecture whereas others only focus on necrosis and/or the degree of nuclear atypia [11–15]. The histopathological assessment of DCIS, and DCIS grading in particular, suffers from substantial inter-observer variability [16]. Besides differences among classification systems, morphological intratumor heterogeneity might also significantly contribute to this inter-observer variability.

In the light of the currently ongoing active surveillance trials for DCIS [17], intratumor heterogeneity might pose a substantial future challenge. If these trials show that watchful waiting is a non-inferior and safe alternative for surgical treatment in selected subgroups of DCIS patients, the decision for active surveillance will be based on the initial breast biopsy [6]. If a DCIS lesion presents with heterogeneous nuclear atypia and comedonecrosis, a biopsy-based decision might potentially underestimate its actual characteristics. This could lead to "undertreatment"; i.e., surgery is not carried out; notwithstanding, the patient would have benefited from an operation.

Since detailed studies on the assessment of heterogeneity of several histopathological features in DCIS are lacking, we aspired to fill this gap in our knowledge. This study aimed to quantify the degree of morphological heterogeneity within a single DCIS lesion, both at the biopsy level and the resection specimen level. We performed a detailed morphological evaluation of six histopathological characteristics, and we compared their distribution between the biopsy and the resection. Inter-slide variability was assessed for each resection.

Materials and methods

Patients and tissue specimens

All patients included in this retrospective study underwent breast-conserving surgery (BCS) or mastectomy at the Cliniques universitaires Saint-Luc (Brussels, Belgium) between 1 January 2015 and 30 April 2019 or at the CHU-UCL Namur-Godinne (Yvoir, Belgium) between 1 March 2004 and 31 December 2018. Inclusion criteria were as follows: (1) both the biopsy and the resection contain pure DCIS without (micro-)invasive component; (2) both the archived biopsy and specimen slides are available for review; (3) surgery was preceded by either core needle biopsy or vacuum-assisted biopsies; (4) at least three formalin-fixed, paraffin-embedded (FFPE) tissue blocks of the resection each contain at least two ductal structures affected by DCIS.

All hematoxylin-eosin (HE)-stained slides were retrieved from the archives of the Department of Pathology of both institutions and were jointly reviewed by two pathologists (CSP and MRVB) using a double-head light microscope. The biopsy slide and three representative slides of the resection were selected for each lesion. If the biopsy comprised more than one slide, the slide with the largest amount of DCIS was selected. If the resection contained multiple FFPE blocks with DCIS, the first three slides were selected for inclusion in this study. Information on patient age at diagnosis, DCIS size, and type of surgery were retrieved from the original histopathological reports. This study was approved by the institutional ethics committee (RETRO-DCIS-13/2019/25JUI/276).

Histopathological assessment

For each patient, the following six histopathological features were assessed in the biopsy and the resection: nuclear atypia, DCIS architecture, comedonecrosis, stromal inflammation, periductal stromal myxoid changes, and calcifications. A histo-score was determined for each feature to allow quantitative assessment of the degree of morphological heterogeneity, as previously described for immunohistochemistry [18]. Heterogeneity was arbitrarily defined as a mean histo-score with a standard deviation (SD) > 10% of the maximum histo-

score. To prevent recall bias during a histopathological assessment, all slides were randomly shuffled and were evaluated independently from one another. The original identification number on the selected slides was hidden by a sticker.

Nuclear atypia (grade) was assessed as a three-tier variable according to the American Society of Clinical Oncology/College of American Pathologists protocol for examination of DCIS specimens [11]. Nuclear atypia was categorized as low, intermediate, or high, on the basis of nuclear size, pleomorphism, chromatin distribution, nucleoli, mitoses, and orientation of nuclei [11]. The histo-score for nuclear atypia was calculated as follows: (% grade 1 nuclei) + (% grade 2 nuclei) ×2 + (% grade 3 nuclei) × 3, with a histo-score ranging from 100 to 300.

The architectural patterns of DCIS include solid, cribriform, papillary, micropapillary, and clinging growth, regardless of comedonecrosis [11, 19]. In the current study, the growth pattern histo-score was calculated as follows: (% clinging) + (% solid) × 2 + (% papillary) ×3 + (% micropapillary) ×4 + (% cribriform) × 5, with a total score ranging from 100 to 500. As the architectural pattern is a nominal instead of an ordinal categorical variable, coefficients were arbitrarily attributed to each architectural pattern. Of note, a single histo-score does therefore not provide useful information regarding a particular architectural pattern. The histo-scores were merely used to investigate the mutual interrelationship between the architectural patterns present in the biopsy slide and those present in the resection slides, since homogeneous lesions likely present similar histo-scores and heterogeneous lesions present with more variable histo-scores.

Necrosis was classified into three categories (no necrosis, single-cell necrosis, and comedonecrosis), as previously described [12]. Comedonecrosis was defined by areas of confluent dirty necrosis, i.e., confluent eosinophilic material, often containing ghost cells and karyorrhectic debris, which is easily detected at low magnification. Single-cell necrosis was defined as punctiform karyorrhectic debris and intraluminal isolated ghost cells. The histo-score for necrosis was calculated as follows: (% no necrosis) + (% single-cell necrosis) ×2 + (% comedonecrosis) ×3, with a total score ranging from 100 to 300.

Intraductal calcifications in DCIS were scored as the percentage of ducts containing calcifications, ranging from 0 to 100 [20]. Periductal stromal changes were preferentially assessed at low magnification and were classified as sclerotic or myxoid stroma, as previously described [21]. Myxoid periductal stroma was defined as loosely arranged collagen fibers, interspersed with an amorphous, slightly basophilic substance [19]. Myxoid periductal stromal changes were quantified as the percentage of ductal spaces surrounded by myxoid stroma, ranging from 0 to 100.

The presence and extent of chronic inflammation in the periductal stroma (regardless of its architecture) were recorded in a semiquantitative manner as previously described and was preferentially assessed at low magnification, distant from the biopsy site in the surgical specimen [19–21]. Ducts "without stromal inflammation" were surrounded by a stroma that was not infiltrated by lymphocytes. Ducts with "mild stromal inflammation" showed a periductal stroma that was infiltrated by a few loosely arranged lymphocytes, but dense lymphocytic aggregates were lacking. Ducts with "moderate stromal inflammation" were surrounded by stroma that contained a moderately dense inflammatory infiltrate: lymphocytic aggregates were present, but lymphoid follicle formation was absent, and assessment of the periductal stroma was not hampered by the density of the infiltrate. Ducts with "extensive stromal inflammation" were surrounded by a dense inflammatory infiltrate, consisting of large lymphocytic aggregates. The density of this infiltrate hampered the assessment of the periductal stromal architecture. Lymphoid follicle formation could be present, but was not a prerequisite [19]. The histo-score for stromal inflammation was calculated as follows: (% no inflammation) + (% mild inflammation) × 2 + (% moderate inflammation) × 3 + (% extensive inflammation) × 4, ranging from 100 and 400.

Statistical analyses

Statistical analyses were performed with IBM SPSS statistics 25.0 (IBM Chicago, IL, USA). For each lesion, the arithmetic mean and the SD of the histo-scores were calculated per histopathological feature. Heterogeneity for a particular histopathological feature was arbitrarily defined as DCIS displaying a histo-score with an SD > 10% of the maximum histo-score. For instance, the maximum histo-score for nuclear atypia is 300, and a DCIS lesion with an SD > 30 for nuclear atypia was considered to show heterogeneity for nuclear atypia. Mann-Whitney *U* tests were performed to investigate whether heterogeneity of a particular feature was associated with DCIS size and patient age at diagnosis. Chi-square tests were performed to evaluate whether morphological heterogeneity was associated with the type of surgery.

Percent concordance was determined per histopathological feature for correlations between each biopsy and the corresponding resection. The Friedman test was applied to investigate overall inter-slide variability per patient. Post hoc Wilcoxon tests with Bonferroni correction were used to assess inter-slide variability in detail, including a comparison of the biopsy with each slide of the subsequent resection, and variability between the different slides of a single resection. All tests were two-sided. Significance levels were set at 0.05 with the exception of more stringent cut-offs ($p < 0.0083$) when the Bonferroni correction was applied.

Results

Patient characteristics and morphological DCIS features

A total of 204 slides of 51 DCIS lesions were histologically assessed, comprising 51 biopsies slides and three slides per subsequent surgical specimen. The mean size of the included lesions was 38 mm (range 11–88 mm). The mean patient age at diagnosis was 56 (range 30–92). This cohort comprised thirty lumpectomy specimens (59%) and 21 mastectomy specimens (41%). Histo-scores were used as an attempt to quantify descriptive histopathological features and to allow for statistical analysis. Descriptive statistics of the histo-scores for each feature are provided in Table 1.

Percent concordance between biopsies and resection specimens

The degree of concordance between each biopsy and its corresponding resection specimen was evaluated. These data are presented in Table 2 and Supplementary Table 1. Percent concordance was lowest for myxoid stromal changes, calcifications and clinging DCIS architecture, with discordance rates of 35, 37, and 45%, respectively. None of the DCIS lesions showed a complete lack of stromal inflammation, although the extent of this stromal inflammatory infiltrate was variable. A total of 12 DCIS (24%) showed discrete stromal inflammation at the biopsy level, whereas this was upgraded to at least moderate stromal inflammation in the resection specimen.

Only two biopsies (4%) did not contain any sign of (single-cell) necrosis, whereas this was present in the resection. When (single-cell) necrosis was detected in the biopsy, this was always confirmed at the resection level. None of the biopsies and none of the resection specimens contained grade 1 nuclei only: the presence of grade 2 nuclei, albeit focally, was never underestimated at the biopsy level. However, eleven biopsies (22%) did not contain grade 3 nuclei, whereas these were present (at least focally) in the corresponding resection specimen.

DCIS architectural patterns were heterogeneously distributed throughout all lesions, with cribriform architecture being the most common and papillary architecture being the least common (Supplementary Fig. 1). Clinging architecture was most frequently undetected at the biopsy level when present in the resection, followed by papillary and solid architecture. The cribriform architecture was present in 201 out of 204 slides evaluated (98%). In 51 biopsies, eighteen (35%) contained only one architectural pattern; 24 biopsies (47%) contained two architectural patterns, 8 biopsies (16%) contained three patterns, and one biopsy (2%) contained four architectural patterns. The resection specimen slides contained one pattern in 37 of 153 slides (24%), two patterns in 64 slides (42%), three patterns in 37 slides (24%), and four patterns in fifteen slides (10%).

Table 1 Descriptive statistics for the histoscores for each histopathological feature in this DCIS cohort (all 204 evaluated slides included, representing 51 biopsies and 51 resection specimens)

	Mean	SD	Min	P25	Median	P75	Max
Nuclear atypia	244	47	105	209	250	290	300
Necrosis	206	58	100	160	210	260	300
DCIS architecture	422	87	30	380	460	500	500
Stromal inflammation	231	62	100	200	218	270	400
Myxoid stroma	19	28	0	0	5	20	100
Intraductal calcifications	16	23	0	0	5	20	100

DCIS, ductal carcinoma in situ; Max, maximum value; Min, minimum value; P25, 25th percentile; P75, 75th percentile; SD, standard deviation

Histo-scores for quantification of heterogeneity

The use of histo-scores, analogous to immunohistochemical evaluation, allowed to quantify the degree of morphological heterogeneity [18]. The distribution of all histo-scores per histopathological feature is shown in Figs. 1 and 2, and morphological heterogeneity is illustrated in Fig. 3. Firstly, we determined whether a DCIS lesion showed heterogeneity for a particular histopathological feature, which was arbitrarily defined as an SD > 10% of the maximum histo-score for that particular feature.

In 51 cases, twenty-four (47%) showed heterogeneity for nuclear atypia, 25 of 51 (49%) showed a heterogeneous DCIS architecture, and 23 of 51 (45%) showed a heterogeneous distribution calcifications. In 51 DCIS, twenty-nine (57%) showed a heterogeneous distribution of necrosis, 22 of 51 DCIS (43%) displayed heterogeneous myxoid stromal architecture, and 21 of 51 DCIS (41%) displayed heterogeneous stromal inflammation.

The presence of heterogeneity was not associated with patient age, DCIS size, or type of surgery except one weak association ($p = 0.048$) of heterogeneity in stromal inflammation with DCIS size (Table 3). DCIS with heterogeneous stromal inflammation tended to be smaller; larger DCIS lesions often showed a homogeneously distributed stromal inflammatory infiltrate. We did not observe any statistically significant association between heterogeneity in nuclear atypia and heterogeneity in other histopathological features ($p \geq 0.05$).

Inter-slide variability in DCIS

Table 4 shows the associations between the histo-scores of the biopsy slide and the histo-scores of the three selected slides of the resection specimen per histopathological feature. Overall, none of the histopathological

Table 2 Number of DCIS lesions with discordant histopathological assessment between the biopsy and the subsequent resection specimen

Histopathological characteristic	Overall discordance *n* (%)	Absence in biopsy *n* (%)	Absence in resection *n* (%)
At least grade 2 nuclear atypia	0 (0)	0 (0)	0 (0)
Grade 3 nuclear atypia	11 (22)	11 (22)	0 (0)
Moderate/extensive stromal inflammation	16 (31)	12 (24)	4 (8)
At least single-cell necrosis	2 (4)	2 (4)	0 (0)
Comedonecrosis	8 (16)	8 (16)	0 (0)
Clinging architecture	23 (45)	21 (41)	2 (4)
Papillary architecture	14 (27)	14 (27)	0 (0)
Micropapillary architecture	13 (25)	13 (25)	0 (0)
Solid architecture	14 (27)	13 (25)	1 (2)
Cribriform architecture	1 (2)	1 (2)	0 (0)
Myxoid stromal changes	18 (35)	17 (33)	1 (2)
Intraductal calcifications	19 (37)	16 (31)	3 (6)

features was statistically significantly different among the four slides of a single patient ($p \geq 0.05$). After a detailed assessment by post hoc Wilcoxon tests, no substantial differences were observed between the morphology of the biopsy slide and the slides of the subsequent resection specimen ($p > 0.0083$; Bonferroni correction). For each histopathological feature investigated, there was no statistically significant inter-slide variability within a single resection specimen ($p > 0.0083$; Bonferroni correction).

Discussion

Cancer evolution is an extremely complex process, characterized by many different aspects. The cancer ecosystem, comprising both the primary tumor microenvironment and distant ecological niches, interacts with a genetically and molecularly diverse cancer cell population [22]. Numerical and structural chromosomal instability and somatic mutagenesis and epigenetics contribute to the development of genetic and molecular intratumor heterogeneity [22]. These mechanisms, including clonal selection under therapeutic pressure, contribute to the development of a heterogeneous cancer cell population. Clonal evolution can occur in a linear or branched manner, as new subclones outcompete the rest of the tumor cell population or divergent subclones emerge independently from one another, respectively [23]. Numerous studies have investigated the molecular heterogeneity in breast cancer, and its role in DCIS progression in particular [24–31]. Heterogeneity in the expression of HER2 and hormone receptors has been extensively evaluated as well [31–33]. Molecular heterogeneity is already present in the preinvasive stage of breast cancer. However, only a few studies investigated the degree of morphological heterogeneity in DCIS, thereby mainly focusing on architectural heterogeneity and heterogeneous nuclear grade [8, 10, 34–36].

In this study, we evaluated the extent of morphological heterogeneity by assessing six histopathological features in a series of 51 large DCIS lesions. Evaluation of morphological heterogeneity in DCIS is especially challenging, as several studies have shown that histopathological assessment of DCIS is characterized by substantial inter-observer variability [19, 20, 37–40]. The present histopathological review was therefore performed by two pathologists using a double-head light microscope. This double-headed evaluation cannot completely neutralize the degree of subjectivity inherent to histopathological evaluation, but we believe it is still more robust than a single pathologist-based opinion. A future computer-assisted evaluation might enable more detailed, objective, and standardized assessment [16].

Without digital image analysis, it is difficult to quantify the degree of morphological heterogeneity in DCIS, especially for multi-categorical parameters such as DCIS architecture or DCIS grade. We therefore applied the histo-score, which is frequently used for a detailed evaluation of both intensity and proportion of immunohistochemical stains [18]. The disadvantage of histo-scores for histopathological assessment is that a single histo-score provides very limited information on a particular histopathological feature. However, it does allow a more objective comparison among the different slides of a single DCIS lesion. One should therefore not focus on the absolute value of a single histo-score but rather investigate their mutual interrelationship within a single DCIS lesion. By applying an arbitrary definition for heterogeneity based on a >10% SD of the maximum

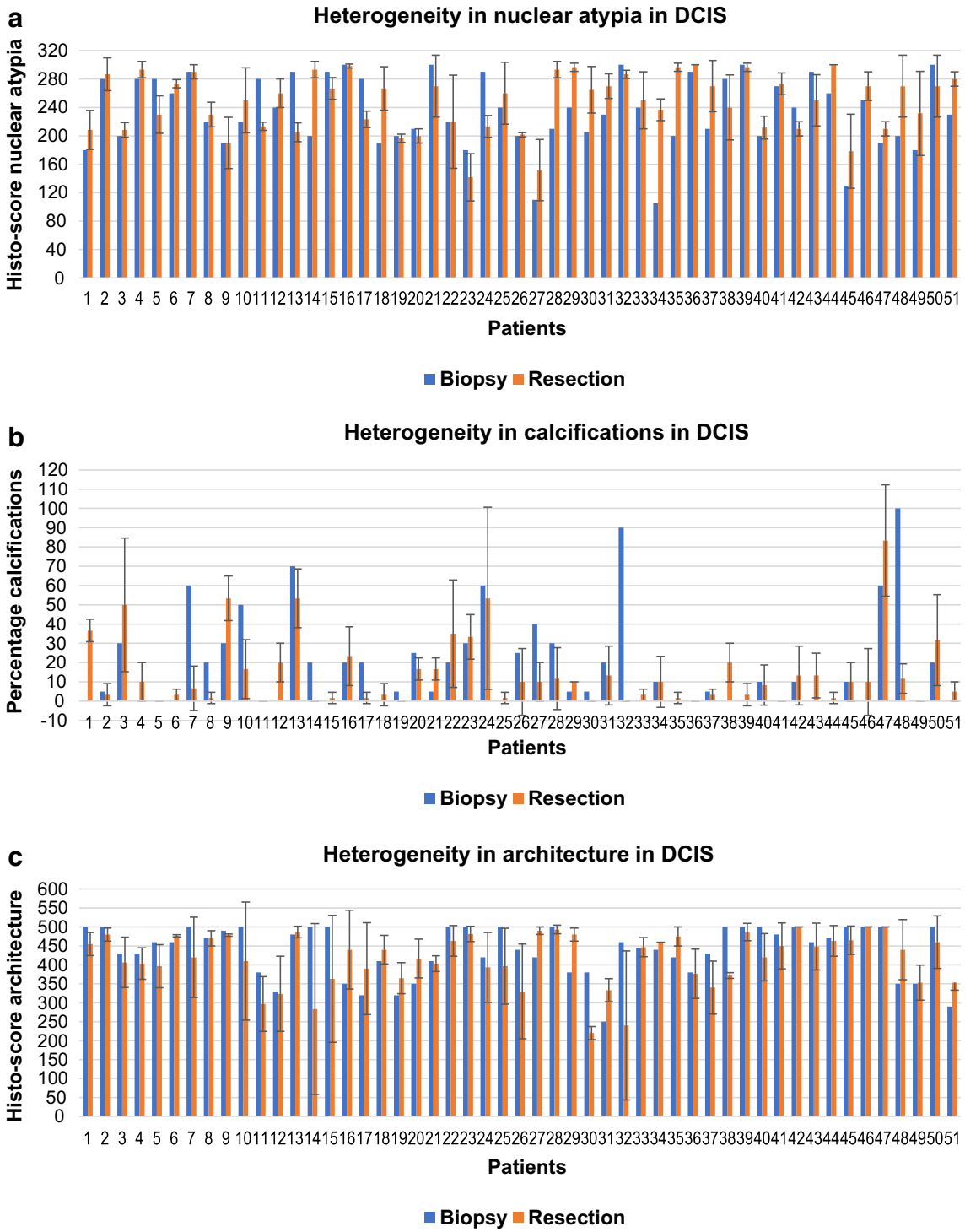

Fig. 1 Bar charts illustrating the histo-score of the biopsy (blue) and the mean histo-score of the resection specimen (orange) per DCIS lesion, for nuclear atypia (**a**), calcifications (**b**), and DCIS architecture (**c**). Whiskers indicate the standard deviation of the mean

histo-score per histopathological feature for four slides of a single DCIS lesion, we were able to identify those DCIS presenting with intra-lesion heterogeneity (i.e., inter-slide variability). Roughly, around half of all DCIS (range 41–57%) showed heterogeneity, depending on the feature evaluated. Stromal inflammation was least often heterogeneously present, whereas necrosis was most frequently heterogeneously distributed between different slides of a single lesion. However, upon comparing the biopsy slide with the three slides of the

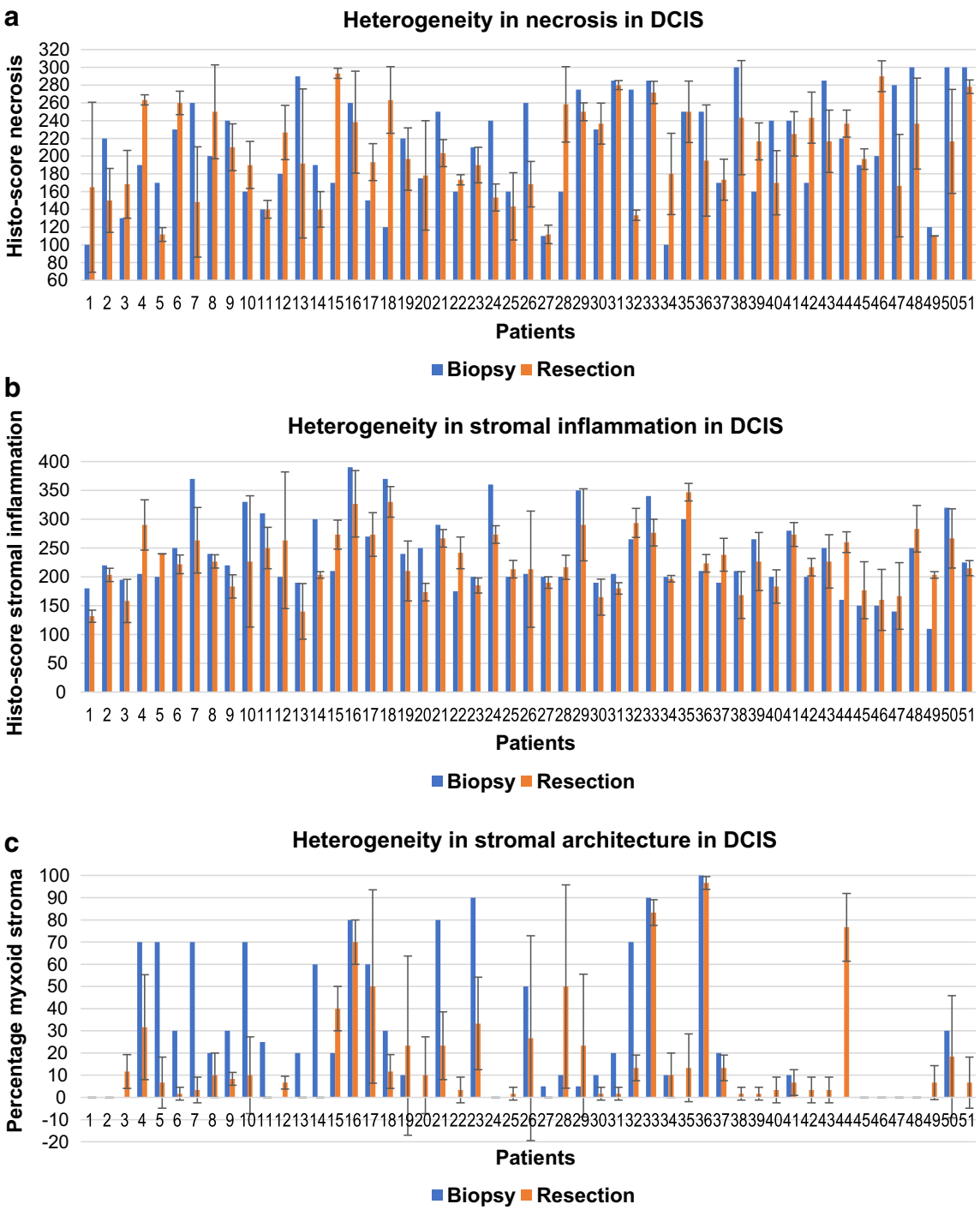

Fig. 2 Bar charts illustrating the histo-score of the biopsy (blue) and the mean histo-score of the resection specimen (orange) per DCIS lesion, for necrosis (**a**), stromal inflammation (**b**), and stromal architecture (**c**). Whiskers indicate the standard deviation of the mean

resection specimen per DCIS lesion, the presence of heterogeneity had no statistically significant impact. Of note, this statement is only valid for the entire evaluated cohort, comprising 51 patients. The lack of statistical significance does not always equal a lack of clinical significance. At the individual patient level, we did observe an impact due to heterogeneously distributed histopathological features. The degree of discordance was highest for clinging DCIS architecture (45%), intraductal calcifications (37%), and myxoid periductal stromal changes (35%). The impact of the heterogeneously distributed necrosis was limited, since only two biopsies lacked necrosis whereas the resection specimen contained at least focal (single-cell) necrosis. The

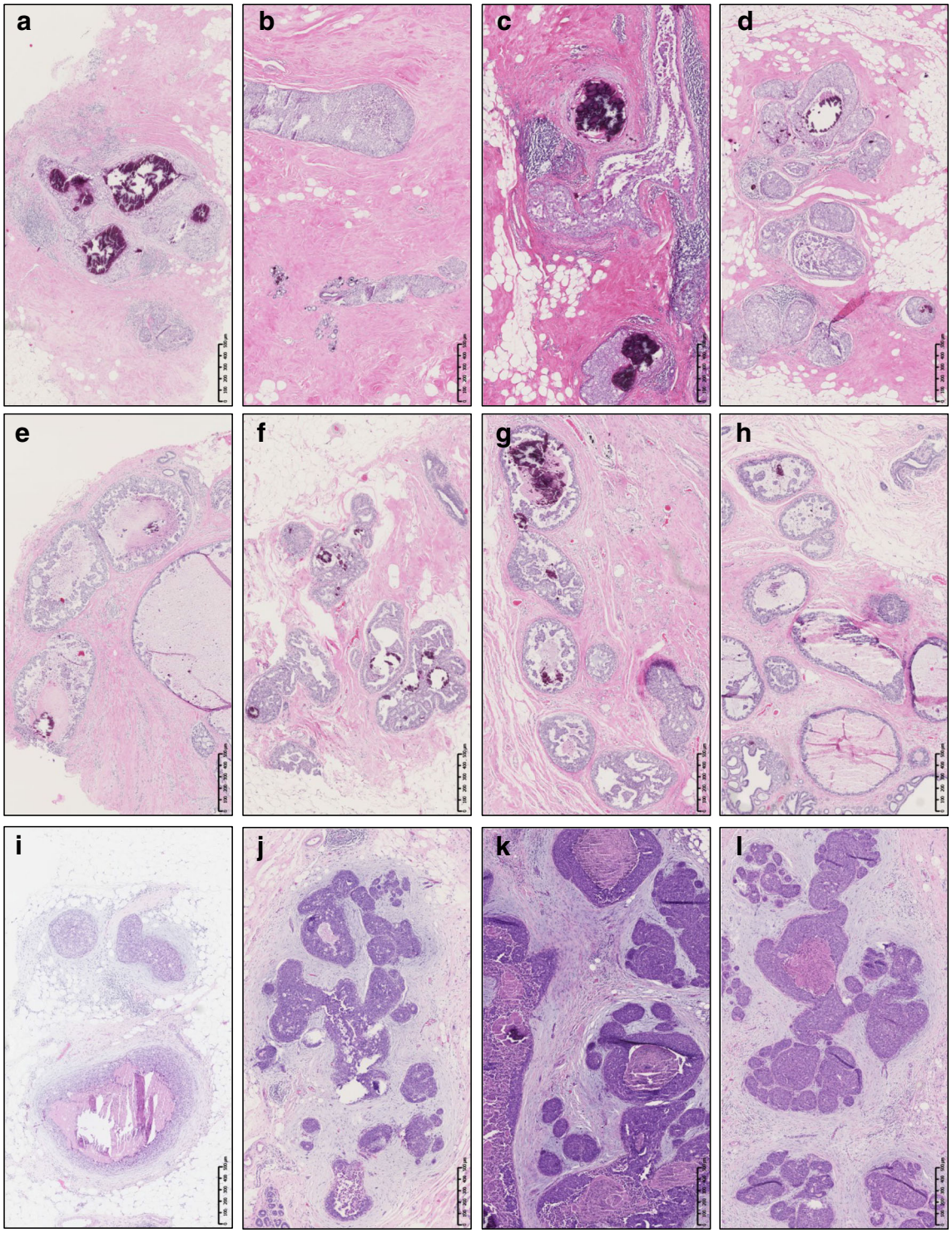

Fig. 3 Photomicrographs of the biopsy slide (**a**, **e**, **i**) and three slides of the corresponding resection specimen of three DCIS lesions at ×50 original magnification. Patient 10 had a DCIS with areas of moderate and high-grade nuclear atypia in the biopsy (**a**) and resection specimen (**b**–**d**), whereas necrosis, the mixed cribriform, and solid DCIS architecture and periductal stromal architecture showed a homogeneous aspect. Calcifications were present in each tissue slide, but the periductal mononuclear infiltrate was absent in one tissue slide of the resection (**b**). Patient 13 had a DCIS with heterogeneous architecture and necrosis in the biopsy (**e**) and the resection specimen (**f**–**h**), but periductal stromal inflammation and stromal architecture were homogeneous. Patient 36 had a DCIS with homogeneous extensive myxoid stroma in the biopsy (**i**) and resection specimen (**j**–**l**)

Table 3 Overview of the *p* values expressing the association between heterogeneity per histopathological feature and DCIS size, patient age at surgery, and type of surgery

	DCIS size°	Patient age°	Type of surgery*
Nuclear atypia	0.623	0.858	0.615
Calcifications	0.596	0.513	0.788
Necrosis	0.118	0.511	0.589
DCIS architecture	0.231	0.351	0.332
Stromal inflammation	0.048	0.674	0.708
Myxoid stroma	0.366	0.447	0.973

DCIS, ductal carcinoma in situ

*Chi-square test

°Mann-Whitney *U* test

impact of the heterogeneously distributed nuclear grade was larger, as 22% of all investigated biopsies did not show grade 3 nuclei, whereas these were present in the resection specimen.

At present, all DCIS patients receive more or less the same treatment. However, this might change in the future, since active surveillance trials are currently ongoing, wherein a standard surgical treatment is compared with "watchful waiting" [17]. Future DCIS treatment might therefore be guided by a histopathological assessment of the biopsy. Nevertheless, our results cannot be generalized to the setting of these trials. To this purpose, we should have limited the analysis to a series of DCIS biopsies presenting grade 1 or grade 2 nuclear atypia without comedonecrosis. We omitted these restrictions, as others already performed such investigations [41–44]. Similarly, we did not aim to investigate the risk of "missing out" on a focus of invasive carcinoma in biopsies containing DCIS only, as former studies already reported on this topic [45–47]. The main goal of this study was to objectify the perception that DCIS is a morphologically heterogeneous disease, as well as to provide a detailed study on the intra-lesion heterogeneity of six histopathological features in a cohort of extensive DCIS. In 1989, Patchefsky et al. observed that extensive DCIS lesions more frequently showed a mixed architectural pattern [34]. It is therefore not surprising to find a similar architectural heterogeneity in the present study cohort, as a minimum of three FFPE tissue blocks containing DCIS was required for inclusion in this study. This setup resulted in a selection of large DCIS lesions, as DCIS with a size < 10 mm were not included. Notwithstanding this potential selection bias, which might have enhanced the high prevalence of heterogeneity in the study cohort, we did not consider it useful to evaluate intra-lesion heterogeneity in small (< 10 mm) DCIS as these lesions are more likely to be removed (almost) completely by vacuum-assisted biopsy.

Conclusion

Our study confirms the presence of morphological heterogeneity in DCIS for each of the six evaluated histopathological features. At the group level, the degree of heterogeneity did not significantly affect the representativity of a biopsy for the entire lesion. At the individual patient level, however, the presence of comedonecrosis, intraductal calcifications, myxoid stromal changes, and high-grade nuclear atypia was underestimated in a minority of DCIS patients. This fact should be kept in mind when taking biopsy-based treatment decisions for patients with large (> 10 mm) DCIS.

Table 4 Overview of all *p* values of the Friedman tests and post hoc Wilcoxon signed-rank tests with Bonferroni correction for the comparison of the histo-scores of each histopathological feature between the initial biopsy and three slides of the subsequent resection specimen

	Overall H-score $	H-score B versus R1 *	H-score B versus R2 *	H-score B versus R3 *	H-score R1 versus R2 *	H-score R1 versus R3 *	H-score R2 versus R3 *
Nuclear atypia	0.050	0.095	0.358	0.021	0.360	0.044	0.363
DCIS architecture	0.462	0.318	0.255	0.060	0.937	0.216	0.158
Calcifications	0.698	0.471	0.700	0.731	0.587	0.742	0.756
Comedonecrosis	0.740	0.217	0.845	0.364	0.268	0.769	0.193
Stromal architecture	0.083	0.046	0.014	0.028	0.986	0.203	0.601
Stromal inflammation	0.132	0.375	0.246	0.107	0.981	0.441	0.224

B, biopsy slide; H-score, histo-score; R1, first slide of the resection specimen; R2, second slide of the resection specimen; R3, third slide of the resection specimen

$ Friedman test with significance level set at < 0.05

*Post hoc Wilcoxon signed-rank test; an association is considered statistically significant if $p < 0.00833$ (i.e., Bonferroni correction for multiple tests on four groups)

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00428-021-03040-6.

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Authors' contributions

- CSP: Data curation, methodology, formal analysis, visualization, project administration, writing of the original draft
- MCN: Data curation, methodology, project administration, writing – review and editing
- MB: Methodology, resources, writing – review and editing
- FPD: Methodology, resources, writing – review and editing
- LF: Methodology, resources, writing – review and editing
- CG: Data curation, methodology, project administration, supervision, writing – review and editing
- MRVB: Conceptualization, data curation, formal analysis, methodology, visualization, writing – review and editing

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FP Duhoux received a postdoctoral clinical mandate (2017-034) from the not-for-profit organization "Foundation Against Cancer" (Brussels, Belgium).

Data availability All data concerning this study can be obtained from the corresponding author upon reasonable request.

Compliance with ethical standards

This study was approved by the Ethics Committee (RETRO-DCIS-13/2019/25JUI/276) of the Cliniques universitaires Saint-Luc (Brussels, Belgium). This study was performed in accordance with the World Medical Association's *Helsinki Declaration* for Human *Studies.*

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

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