Standard operating procedures (SOP) for histopathological assessment of invasive lobular carcinoma

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Invasive breast carcinomas are evaluated on hematoxylin and eosin (H&E)-stained slides with or without adjunctive immunohistochemistry (IHC) for the diagnosis of invasive lobular carcinoma (ILC) (Figure1). Cases that fulfill the histologic criteria for classic ILC on H&E-stained sections as defined by the WHO [2] can be diagnosed as ILC without additional investigations (Table 1).

Table 1. Histological criteria for classic ILC:

A proliferation of small cells that lack cohesion and appear individually dispersed throughout a fibrous connective tissue or arranged in single file linear cords that invade the stroma or adipose tissue. These infiltrating cords frequently present a concentric pattern around normal ducts. There is often little host reaction or disturbance of the background architecture. The neoplastic cells have round or notched ovoid nuclei and a thin rim of cytoplasm, with occasional intracytoplasmic vacuoles, often harboring a central mucoid inclusion.

Cases that do not fulfill the criteria of classical ILC but exhibit one or more of the features listed in table 2 should be further studied by (figure 1), to diagnose non-classical ILC variants like pleomorphic, histiocytic, alveolar, trabecular, solid and extracellular mucin producing ILC. These features are based on well-known features of ILC described in literature[3]. Of note, atypical lobular hyperplasia (ALH) and/or lobular carcinoma *in situ* (LCIS) further lumped here as lobular neoplasia (LN), are included, and are considered a non-obligate precursor of ILC. Its presence is not required for the diagnosis but acknowledged as a desirable criterion by the WHO [2]. If LN is present and shares the same cytonuclear morphological features as the invasive carcinoma, this could be an additional element in favor of ILC[2].

It is important to note that some ILC demonstrate a combination of different morphological patterns, including classic and one or more variant patterns.

Table 2. Histopathological criteria that point to ILC:

- Linear infiltration and/or single cell infiltration of tumor cells
- Circumferential infiltration of tumor cells around ducts
- Infiltration of adipose tissue without stromal reaction
- Inter-cellular dyscohesion
- Intracytoplasmic vacuoles
- Uniform, monomorphic nuclei
- Single cells or small, loosely cohesive cells groups in stromal mucin pools
- Associated ALH and/or LCIS
- Architecture score 3 in the Nottingham grading system, in conjunction with any of the above

E-cadherin immunohistochemistry

A key biomarker of ILC and LN is the lack of expression of E-cadherin, a central protein in the adherens junction that normally keeps epithelial cells together.

Technique:

- Immunohistochemistry using the clones NCH-38 (Agilent) or ECH-6 (Zytomed), according to the protocol attached (Appendix A.1 and A.2 respectively) is recommended.
- The recommended practice is to add an external control (normal peri-tumoral terminal ductulo-lobular units) on the slide if internal normal breast parenchyma is absent.

Interpretation of E-cadherin IHC:

- Three main patterns may be recognized during microscopic investigation:
    - Normal: complete circumferential plasma membrane expression showing the same intensity and pattern of the normal internal or external control.
    - Negative/absent: plasma membrane expression is not detectable.
    - Aberrant: all expression patterns other than normal (i.e. 'fragmented, beaded membranous staining', 'a perinuclear Golgi-dot like pattern', 'cytoplasmic staining' etc.) [3,5,6].
- The internal control is evaluated first to confirm adequate staining and to appreciate the intensity of staining.
- When E-cadherin membrane staining is absent from the tumor cells in combination with retained plasma membrane expression in internal or external control, the diagnosis of ILC is made.
- In cases demonstrating an aberrant E-cadherin staining pattern in conjunction with morphology suggestive of ILC, additional IHC is recommended with β-catenin (Clone 14) and/or p120-catenin (Clone 98).

- The integrative flow-chart suggesting stepwise use of ancillary IHC is shown in figure 1.

Catenin immunohistochemistry

Catenin's (alpha- beta- and p120-catenin) bind with E-cadherin to the internal aspect of the cell membrane to form the cadherin-catenin complex. This complex is essential for the intercellular epithelial junctions and regulation of the cytoskeleton.

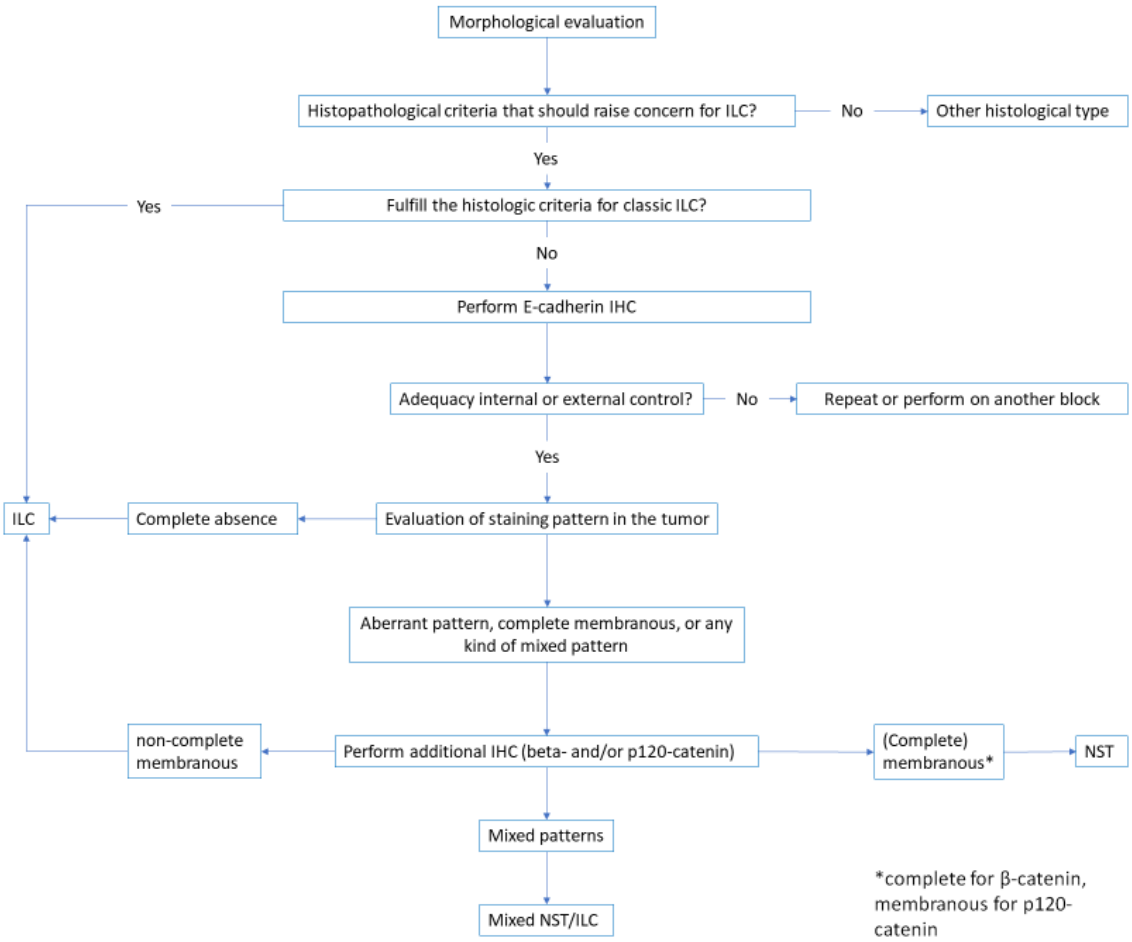
Technique:

IHC staining protocols for β-catenin (Clone 14) and p120-catenin (Clone 98) are included in appendix A.3 and A.4 respectively.

Interpretation:

- Localization of β-catenin and p120 catenin at the plasma membrane is compatible with invasive breast cancer of no special type IBC-NST.
- A cytoplasmic p120-catenin or incomplete membranous/cytoplasmic/absent β-catenin expression is compatible with ILC.
- In cases where there is an admixture of (complete) membranous and non-membranous catenin staining patterns, the diagnosis of a mixed IBC-NST/ILC can be rendered in conjunction with morphology. Additional research is on-going to better understand these complex phenotypes.
- The integrative flow-chart suggesting stepwise use of ancillary IHC is shown in figure 1.

Figure 1: Schematic overview of histopathological review



Appendix: staining protocols

A.1. E-cadherin clone NCH-38 staining protocol

Primary antibody: E-cadherin

- Clone: NCH-38; mouse / IgG1, kappa
- Manufacturer: Agilent, Santa Clara, CA, USA
- Dilution: RTU or 1/50 if concentrate

Staining device: Dako Omnis

Protocol used in cad-E-lac study (S66045, University hospitals Leuven). The protocol is adapted from NordiQC run 53. FFPE tissue sections of 3-4µm thick are made and put on coated slides. The slides are subsequently dried in oven for 30 min. at 70 °C. Further steps are performed on the automated stainer.

A.2. E-cadherin clone ECH-6 staining protocol

Primary antibody: E-cadherin

- Clone: ECH-6; mouse / IgG1
- Manufacturer: Zytomed Systems GmbH, Berlin, Germany
- Dilution: 1:600

Staining device: Ventana Benchmark ULTRA

Protocol used in cad-E-lac study (S66045, University hospitals Leuven). The protocol is adapted from NordiQC run 53. FFPE tissue sections of 3-4 µm thick are made and put on coated slides. The slides are subsequently dried in oven for 30 min. at 70°C. Further steps are performed on the automated stainer.

A.3. β-catenin, clone 14 staining protocol

Primary antibody: β-catenin

- Clone: Clone 14; mouse / IgG1
- Manufacturer: BD Biosciences, New Jersey, USA
- Dilution: 1/75

Staining device: Ventana Benchmark ULTRA

Protocol used in cad-E-lac study (S66045, University hospitals Leuven). The protocol is adapted from Christgen *et al.* 2020. FFPE tissue sections of 3-4µm thick are made and put on coated slides. The slides are subsequently dried in oven for 30min. at 70°C. Further steps are performed on the automated stainer.

A.4. P120-catenin, clone 98 staining protocol

Primary antibody: p120-catenin

- Clone: Clone 98; mouse / IgG1
- Manufacturer: BD Biosciences, New Jersey, USA
- Dilution: 1/250

Staining device: Ventana Benchmark ULTRA

Protocol used in cad-E-lac study (S66045, University hospitals Leuven). The protocol is adapted from Christgen *et al.* 2020. FFPE tissue sections of 3-4 µm thick are made and put on coated slides. The slides are subsequently dried in oven for 30min. at 70 °C. Further steps are performed on the automated stainer.

References

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