What is the clinical value of isolated tumor cells and micrometastases in sentinel nodes? – Pathology issues

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No conflict of interest
CAN WE FIND THEM?
1995 statement

Improved Axillary Staging of Breast Cancer with Sentinel Lymphadenectomy

Armando E. Giuliano, M.D., Paul S. Dale, M.D., Roderick R. Turner, M.D., Donald L. Morton, M.D., Sheila W. Evans, R.N., M.S., and David L. Krasne, M.D.

Sentinel lymphadenectomy with multiple sectioning and immunohistochemical staining of sentinel nodes increases the accuracy of axillary staging in breast cancer and can identify significantly more patients with lymph nodes metastases, especially micrometastases, than can ALND with routine histopathologic processing of lymph nodes.

**Percentage of nodal involvement**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Nodal status</th>
<th>Percentage of nodal involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>N0</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>N+</td>
<td>71</td>
</tr>
<tr>
<td>SNB</td>
<td>N0</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>N+</td>
<td>58</td>
</tr>
</tbody>
</table>

N=134 (AD)
N=162 (SNB)

13% more N+
28% more micrometastasis
Micrometastatic cases (SEER) (cleaned data)

By courtesy of Vincent Vinh-Hung (AZ-VUB, Jette, Belgium) and SEER
Upstaging of BC via SNB

There is a very wide variation in the methodology, but a combination of "multilevel" assessment and IHC may "upstage" BC in 9 - 47% of the cases.

Upstaging vs "standard” / "conventional” histopathology

CAN WE FIND THEM?

YES
CAN WE FIND THEM ALL?
There were 123 somewhat different protocols used in the 240 pathology departments dealing with SNs.

Multiple levels are assessed in 88% of the labs via sectioning or/and slicing.

Immunohistochemistry was used routinely in 71%.
Trends and possibilities in SN histopathology

SLN assessment

- Standard histopathology
- Enhanced histopathology

- Limited sampling with or without IHC
- Thorough (systematic) sampling
**The Santa Monica protocol**

**(1995 & 2004)**

Halving of the lymph node in its hilar plane, which they claim to be the most likely site of metastases.

- 1 HE frozen
- 1 HE paraffin
- 1 CK IHC

Separated by 40-200 μm from the initially assessed level (per half)

Rather large proportion of the SN not investigated – LIMITED SAMPLING + IHC
Multilevel assessment
A combination of slicing and step sectioning

3 slices step sectioned at 250 μm
Full thickness assessment of SNs

Patients

Central cross section

1

2a*

3*

4*

5

6*

7*

8

9a*

9b

10

11a

11b

12

13a

13b

14a*

14b

15

16

17

18a

18b

19*

20

21

Scale: 5 levels at 50-100 microns

Complete step sectioning (50-100μm) + IHC /300-600 μm

The impact of methods...

• The more we look for, the more we find, and the majority of the extra yield belongs to the category of micrometastasis / ITC...

• ... but, histopathology is not able to identify all SLNs involved by low volume deposits.

• It can be tailored to identify "virtually all" micrometastases, but it is unsuitable to find all ITCs.

• Intraoperative microscopy often fails with micrometastases.
Meta-analysis of 31 studies on imprint cytology of SLNs

- Pooled sensitivity: 63% (95%CI: 57-69%)
- Pooled specificity: 99% (95%CI: 98-99%)

- Pooled sensitivity for macrometastases is different from that for micrometastases: 81 vs 22 %.

Meta-analysis of 47 studies on frozen section of SLNs

- Pooled sensitivity: 73%
- Pooled specificity: 100%

- Mean sensitivity for macrometastases is different from that for micrometastases: 94 vs 40 %.

The addition of IHC to frozen sections

- Center A (IHC on FS)
  - 2 levels at 200 microns HE + IHC
  - Final: 2 levels at 500 microns: HE + IHC
  - 335 patients:
    - 31% positive intraoperatively
    - 94% (75/80) MAC found intraoperatively
    - 72% (18/25) MIC found intraop
    - 77% (10/13) ITC found intraop

- Center B (no IHC on FS)
  - 2 levels at 200 microns HE only
  - Final: 2 levels at 500 microns: HE + IHC
  - 336 patients:
    - 21% positive intraoperatively
    - 91% (63/69) of MAC found intraoperatively
    - 17% (6/35) MIC found intraop
    - 8% (1/12) ITC found intraop

Molecular assays for intraoperative assessment of SNs

- **In house assays**
- **BLN assay** (Veridex) MG & CK19 dual marker quantitative RT-PCR – positive experiences (withdrawn from market)
- **OSNA** (Sysmex) CK19 demonstration one step nucleic acid amplification byproduct
OSNA vs HIST (alternative slices) Validation studies

<table>
<thead>
<tr>
<th></th>
<th>HIST-</th>
<th>HIST+</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSNA-</td>
<td>2935</td>
<td>50</td>
<td>2985</td>
</tr>
<tr>
<td>OSNA+</td>
<td>92</td>
<td>554</td>
<td>646</td>
</tr>
<tr>
<td>All</td>
<td>3027</td>
<td>604</td>
<td>3631</td>
</tr>
</tbody>
</table>

- **OSNA (HIST GS)**
  - SENS 91.7%
  - SPEC 97%
  - FNR 8.3%
  - FRR 1.7%

- **HIST (OSNA GS)**
  - SENS 85.8%
  - SPEC 98.3%
  - FNR 14.2%
  - FRR 3%

FRR: FNs/all testing negative

Cserni G. J Clin Pathol 2012
Low volume metastases in validation studies

- MIC 107
- OSNA+ 176
- Concordant MIC/OSNA+ 37
- MIC overrated as OSNA++ 29
- MIC underrated as OSNA- 41
- OSNA+ overrated as MAC 38
- OSNA+ underrated as HIST-/ITC 101

OSNA identifies more low-volume metastases. (More thorough sampling and STANDARDIZED methods)

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MICROSCOPIC PATHOLOGY

• The role of pathological examination of sentinel nodes in the treatment of breast cancer:
  - to identify patients who harbor metastasis in the SN, and allow ALND in the same operative session (intraoperative)
  - to improve nodal staging by better identifying a rather homogeneous pN0 group potentially harboring occult metastases smaller than a given size (permanent serial sections and IHC)
(Exclusive) molecular examination of sentinel nodes would result in:
- standardized methods
- the identification of all CK19+ metastases (including all micrometastases) in the SN, and allow ALND in the same operative session (intraoperative only)
- the improvement of nodal staging by finding virtually all patients in the pN+ group (without any morphological correlates, such as extracapsular spread, size, location… other nodal changes)
CAN WE FIND THEM ALL?

NOT WITH MICROSCOPY
CAN WE DISTINGUISH BETWEEN MICROMETASTASIS AND ITC?
Micromets are not homogeneous

- NSN+ $\leq 10\%$ if $pN1mi \leq 1.3$ mm and NSN+ $>10\%$ if $pN(sn)>1.3$ mm (n=12)
- $pN1mi \leq 1$ mm or $>1$ mm $8\%$ vs $28\%$ NSN+ (n=62)
- $pN1mi \leq 1$ mm or $>1$ mm $21\%$ vs $37\%$ NSN+ (n=84)
- $pN1mi \leq 1$ mm or $>1$ mm $16\%$ vs $36\%$ NSN+ (n=109)
- To be fair there is also opposing literature…
NOT HAPPY with TNM6 definitions
ITC vs micrometastasis distinction

Size
Localization
Stromal reaction/desmoplasia
Capsular penetration
Mitoses
Lobular type
Distribution (homo- vs heterogeneous)

A French study concludes…

About one half of the cases (n=337) with small nodal involvement can be unanimously classified on the basis of the current TNM definitions.

Algorithm to distinguish between MIC and ITC (TNM7)
CAN WE DISTINGUISH BETWEEN MICROMETASTASIS AND ITC?

NOT OPTIMALLY, BUT THIS HAS IMPROVED
CAN PATHOLOGY BE ADOPTED TO POTENTIAL CLINICAL NEEDS?

I BELIEVE, YES
Do we need to identify micromets?

- If the answer is no:
  - No need for OSNA, IC and FS are of sufficient sensitivity
  - No need for steps < 2 mm in serial sections

- If the answer is yes:
  - OSNA is more sensitive in this setting than IC or FS (unless FS is complemented with IHC), but does not allow to classify metastases according to currently defined standard categories.
  - If there are permanent sections, steps < 2 mm would be adequate
ALND not considered as regional treatment option*

Only macrometastases considered for further treatment decisions

Micrometastases also considered for further treatment decisions

No intraoperative assessment & levels examined by HE at 2 mm intervals

No intraoperative assessment & levels examined by HE at 0.2 to 1 mm intervals

IHC for equivocal cases (or routinely)

ALND considered as a regional treatment option.

- Only if SLN+ by macrometastasis
- Even if SLN+ by micrometastasis

**Intraoperative frozen sections** (imprint cytology) (quantitative molecular assay e.g. OSNA) processed intraoperatively and minimal tissue left for microscopic verification.

- Quantitative molecular assays

**Permanent HE sections** at 2 mm intervals

- Rapid IHC

**Frozen sections** (imprint cytology) at 0.2 to 1 mm intervals
- IHC for equivocal cases (or routinely)