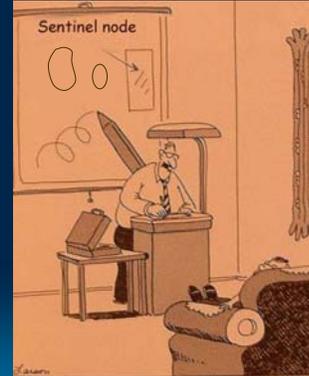


Pathological Assessment of Sentinel Lymph Nodes

Dr. Kathy Ceballos
Anatomical Pathologist
BCCA, Vancouver site



Eventually, everyone came to dread pathology lectures over all other forms of punishment.

Pathological assessment of sentinel lymph nodes (SLN)

- Team approach
- Pathological protocols and rationale
- Potential for false positives
- Macrometastases, micrometastases and isolated tumor cells in breast carcinoma
- Intra-operative assessment
- Impact of SLN biopsies on pathology dept

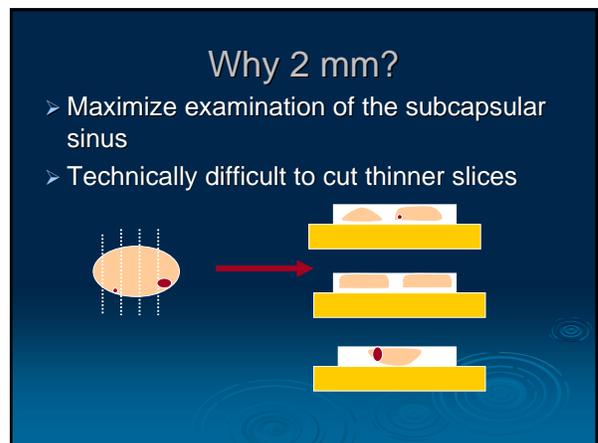
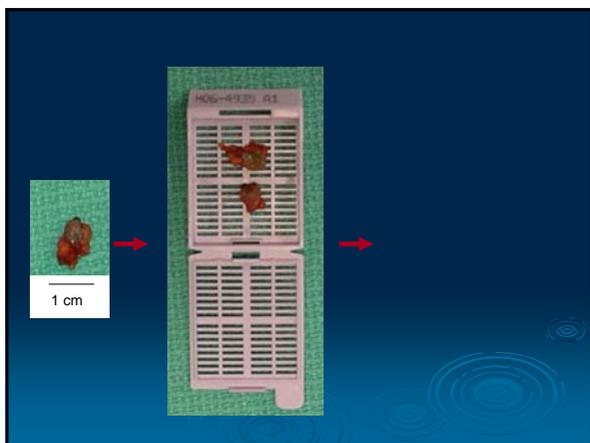
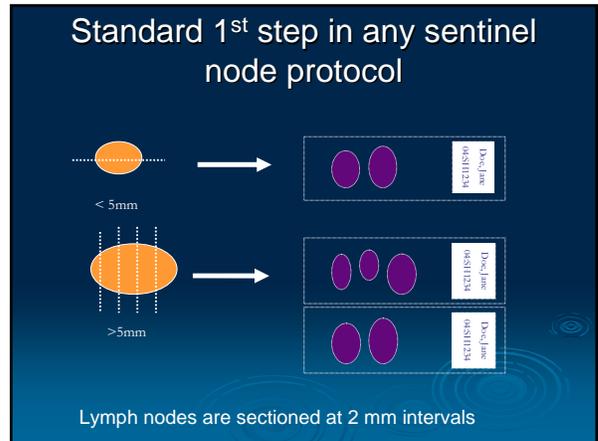
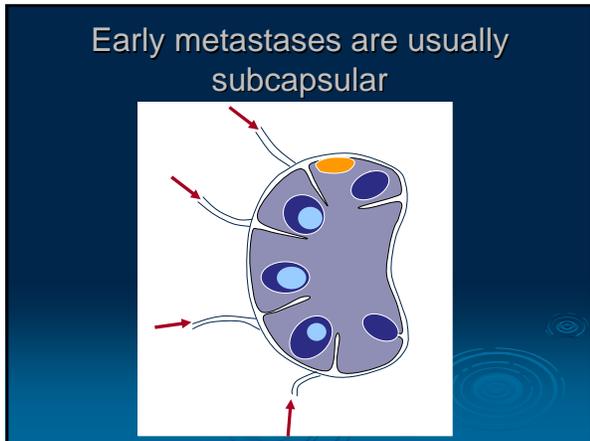
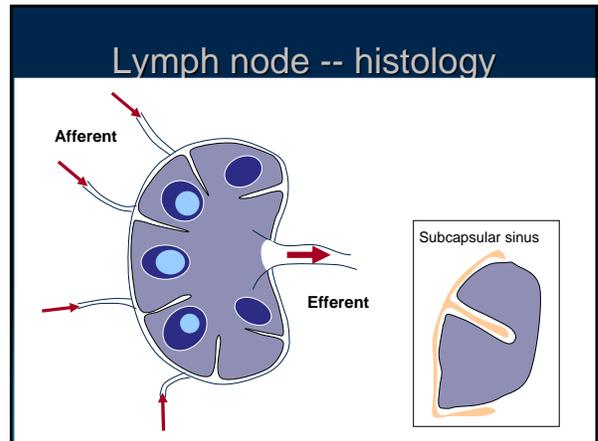
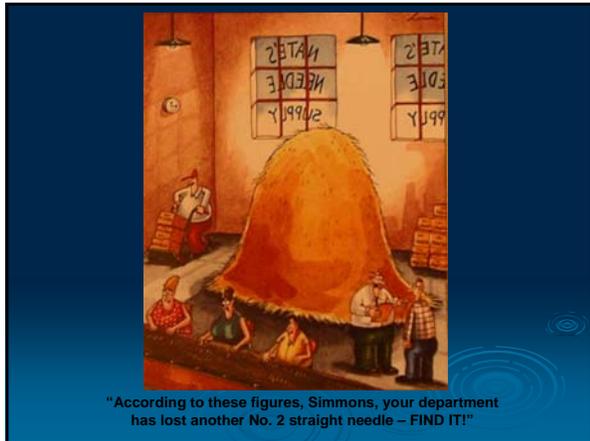
Team Approach

- Critical to coordinate nuclear medicine, surgery and pathology BEFORE sentinel lymph node surgery is instituted
- Variability in protocols between institutions
- Protocol MUST be standardized within an institution
 - Predictive value is likely 'team' specific
 - During initial cases completion axillary dissection after sentinel node biopsy

Pathological Protocols

Sentinel Lymph Node protocol

- **Goal:** Identify clinically significant metastatic deposits in sentinel lymph nodes
- **Requirements:**
 - A sensitive but practical method of examination
 - Criteria to determine which metastases are meaningful – disease specific



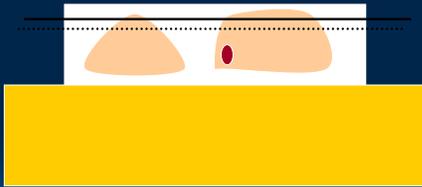
If there are no metastases on initial slides from a **sentinel node**....

- Additional slides cut:
 - Breast carcinoma: 3 H&E levels
CAM 5.2 IHC
 - Melanoma: 3 H&E levels
S-100 and melanA/HMB45 IHC
 - Cervix, vulvar Ca: 3 H&E levels
HMW cytokeratin IHC
- A total of 5 or 6 slides per block + control slides

Sentinel node RT-PCR

- RT-PCR converts RNA to DNA
- Has been used to identify tyrosinase (melanoma), keratins (carcinoma)
- **Dramatic** upstaging of patients
 - does not correlate with outcome
- No role outside of the research setting

How effective is the protocol?



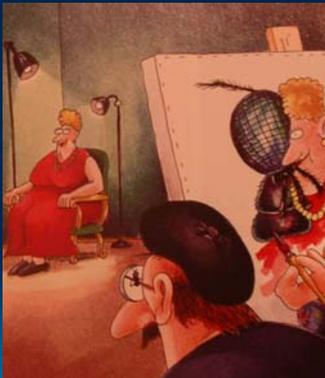
If 100 um between sections, examine top 0.5 mm
If 50 um between sections, examine top 0.25 mm
(Assumes 5 levels, with 1,3,5 for H&E, 2 and 4 reserved for IHC)

Annals of Surgical Oncology (© 2006)
DOI: 10.1245/s10434-006-9084-y

Differences in Sentinel Lymph Node Pathology Protocols Lead to Differences in Surgical Strategy in Breast Cancer Patients

Marieke J. Bolster, MD,¹ Peter Bult, MD,² René F. M. Schapers, MD PhD,³
Jos W. R. Meijer, MD,⁴ Luc J. A. Strobbe, MD PhD,⁵ Charles L. H. van Berlo, MD PhD,⁶
Jean H. G. Klinkenbijn, MD PhD,⁷ Petronella G. M. Peer, PhD,⁸ Theo Wobbes, MD PhD,¹
and Vivianne C. G. Tjan-Heijnen, MD PhD⁷

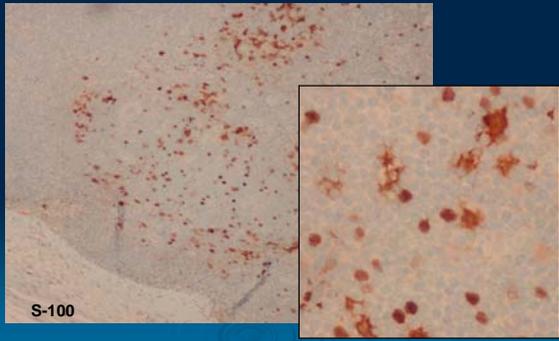
- 541 pts underwent SLN bx at 4 hospitals
 - 3 used 3 levels, 4th used ≥ 7
 - Completion ALND: 29% vs 66.3%
 - Negative completion ALND: 19.3% vs 52.4%



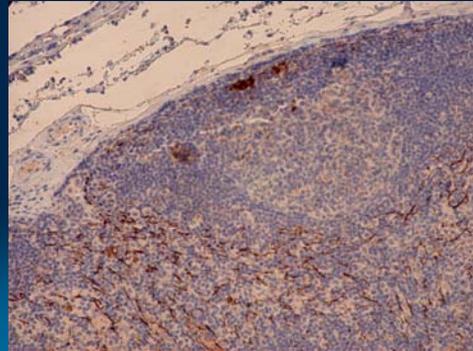
Potential for false positives

- Immunohistochemistry:
 - non specific staining
 - Cross reactivity – especially dendritic cells
- Benign inclusions
 - Axilla – benign breast tissue, nodal nevi
 - Head and Neck – benign squamous inclusions, thyroid tissue
 - Pelvis – Mullerian tissue
- Mechanical transport of benign epithelium
 - Breast tissue from biopsy or injection site massage
 - Mesothelial cells in pelvic nodes

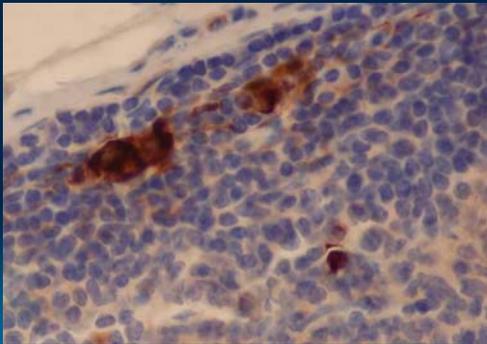
False positive – dendritic cells



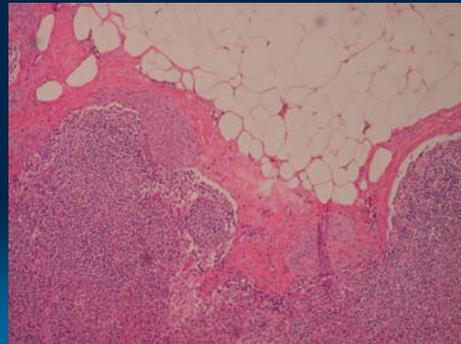
False positive – keratin



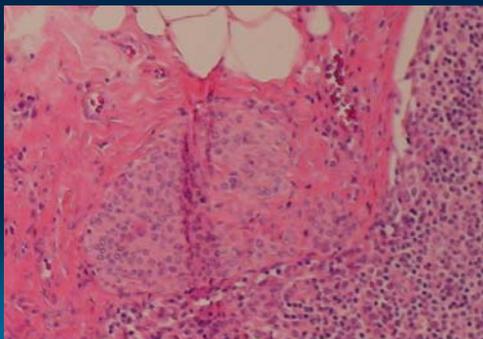
False positive -- keratin



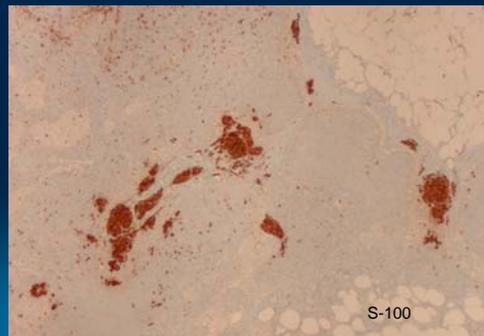
False positive



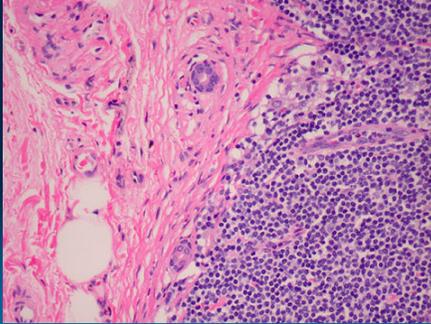
False positive—capsular nevus



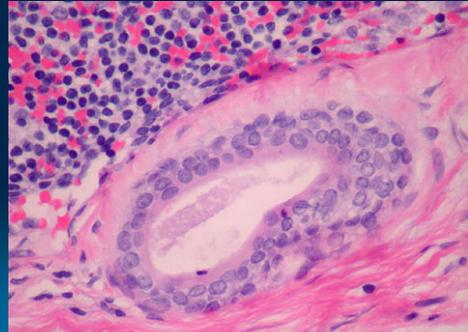
False positive—capsular and intranodal nevus



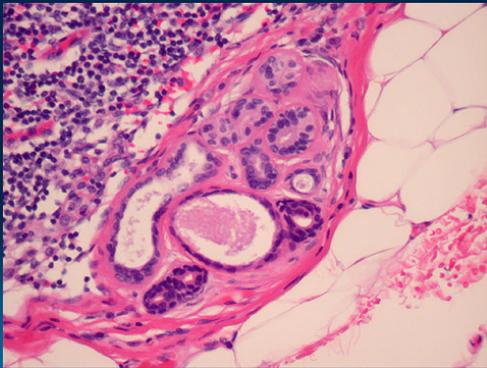
False positive diagnosis – normal breast tissue inclusion



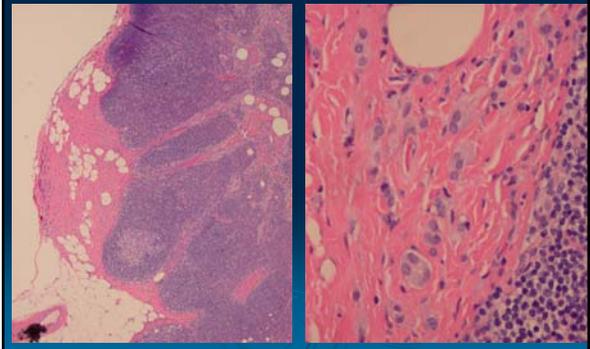
False positive diagnosis – normal breast tissue inclusion



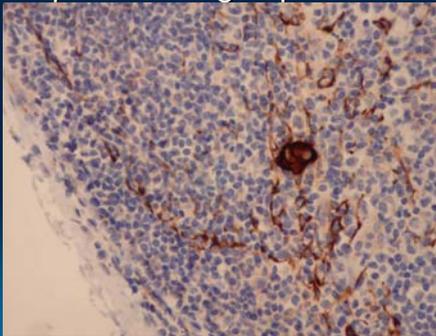
Inclusion or Metastasis?



Inclusion or Metastasis?



Isolated tumor cells or mechanical transport of benign epithelium ?



Breast Carcinoma

Macrometastases,
Micrometastases and Isolated
tumor cells (ITCs)

Controversy in breast carcinoma:

What is significant?

- Macrometastases: > 2 mm
- Micrometastases: 0.2 – 2 mm
- Isolated tumor cells: <0.2 mm
- Criteria are now being defined, but very small metastases are not predictive of non-sentinel node involvement or adverse prognosis

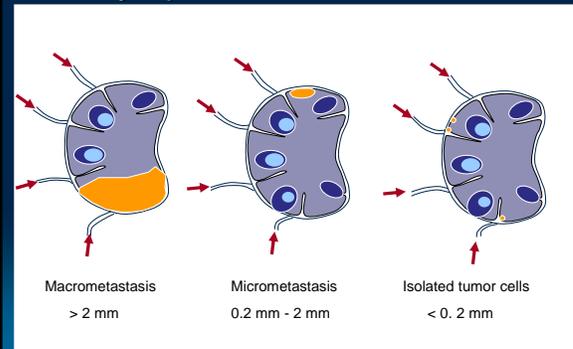
AJCC sixth edition (2002)

- pN0(i+): Isolated tumor cells
 - No individual cell clusters > 0.2mm
 - Detected with routine stains and/or IHC
- pN1mi: Micrometastases
 - Deposit measures <2mm but >0.2 mm
- pN1: 1 -3 positive lymph nodes with at least 1 deposit measuring > 2 mm

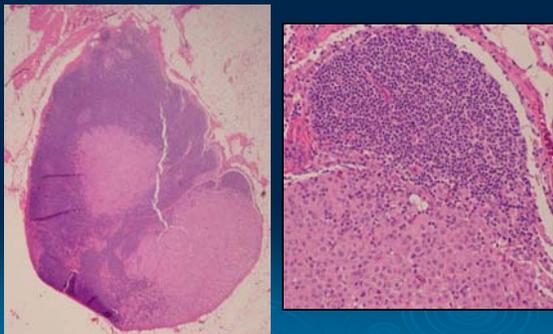
Nodal Staging

- Once there is a deposit > 2mm the size of other deposits is immaterial for staging
 - 4 positive lymph nodes – largest deposit 3mm, 3 nodes ITCs only
 - ➔ pN2
 - 4 positive lymph nodes – all nodes show ITCs
 - ➔ pN0(i+)

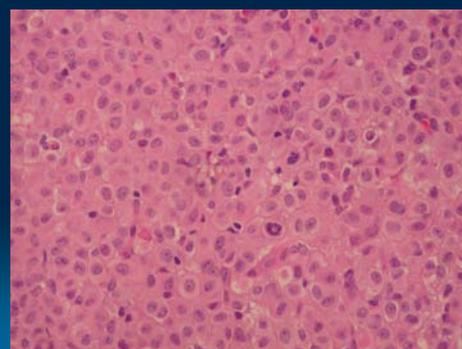
Lymph node metastases



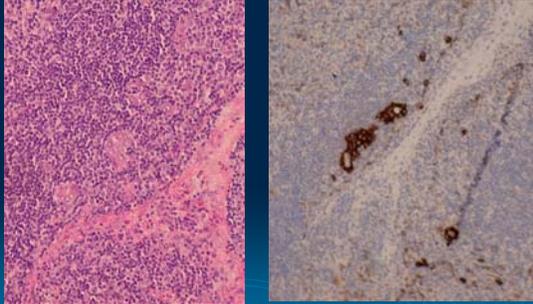
Macrometastasis -- >2 mm



Macrometastasis -- > 2mm



Isolated tumor cells – pN0(i+)



Micromets and ITCs: Adjuvant treatment

- Retrospective study --214 consecutive cases of node negative breast cancer
- Mean follow-up 8 yrs, 5% had adjuvant systemic treatment
- Re-examined lymph nodes with further H&E levels and IHC
 - 29/214 cases (14%) had metastases
 - 2 cases pN0(i+) – ITCs
 - 27 cases pN1mi – micrometastases

Kahn et al, *Breast J* Jul-Aug 2006

Micromets and ITCs: Adjuvant treatment

- No association between occult metastases and
 - Disease free interval
 - Disease specific survival
- Conclusion
 - Systemic adjuvant therapy should **NOT** be given on the basis of micrometastases or ITCs

Kahn et al, *Breast J* Jul-Aug 2006

Micromets and ITCs: Completion axillary dissection

- Retrospective evaluation of 2150 breast Ca pts with SLN biopsy
 - 649 (30%) + node --148 micromets, 105 ITCs
 - 106/148 full axillary lymph node dissection
 - 20 (19%) additional mets (4 pN1mi, 10 pN1a, 6 pN2a)
 - 7 received adjuvant therapy based on findings
 - 54/105 full axillary lymph node dissection
 - 4 (8%) additional mets (2 pN1mi, 2 pN0i+)
 - 0 received adjuvant therapy based on findings

Cancer Aug 2006

Micromets and ITCs: Completion axillary dissection

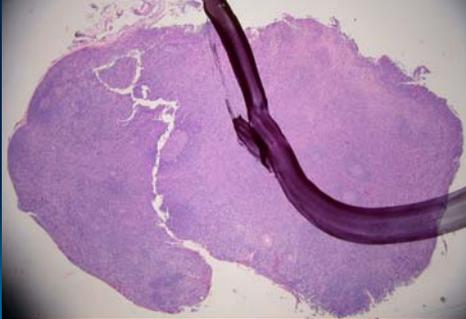
- CONCLUSION: Axillary lymph node dissection indicated in patients with micrometastases on SLN bx
- Pts with ITCs – 4% had macrometastases
 - If don't do lymph node dissection then there is a resulting 'false negative' rate in 4% of 105/2150 = 0.2% of patients who underwent SLN
 - False negative rate 5-10% → 5.2-10.2 %

Cancer Aug 2006

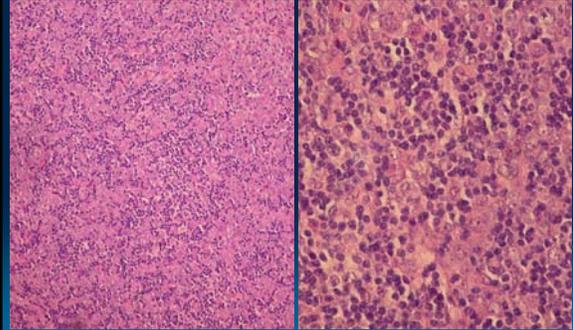
Why are we doing IHC in SLN for breast cancer?

- If node is sectioned at 2 mm intervals then all macrometastases will be identified on the initial level.
- Experienced pathologists will identify micrometastases and ITCs on routine stains
 - IHC – helpful quality check if SNL protocol is infrequent
 - Very useful in the setting of lobular carcinoma

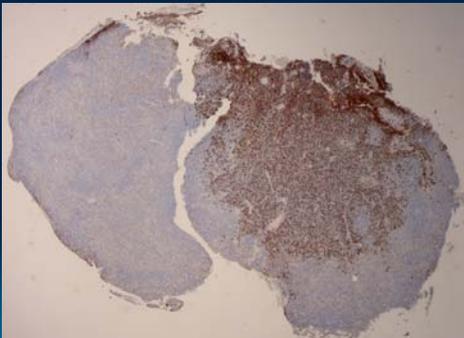
Role of immunohistochemistry



Role of immunohistochemistry



Role of immunohistochemistry



Metastases in SLN detected by IHC were associated with additional mets in completion axillary dissection in 24% of cases (Cserrni et al 2006)

Future protocols

- IHCs likely eliminated from breast protocol, except in cases of lobular carcinoma
- If micrometastases (pN0mic, >0.2 mm) are determined to be significant, likely levels will continue to be done but spaced further apart
- Other protocols will be modified as significance of small mets in other malignancies is determined
- Hopefully will be standardized!

Intraoperative assessment of sentinel nodes

Intraoperative assessment of sentinel nodes

- Ideal
 - accurate, inexpensive and speedy intraoperative assessment of SNL
 - Proceed to full lymph node dissection
- Reality
 - Full protocol cannot be performed quickly or inexpensively
 - Current methods are insensitive

Intraoperative assessment of sentinel nodes

- Touch preps – cytology
 - Only examines cells from cut section
 - Low numbers of malignant cells will likely be missed
 - Theoretical risk of false + diagnosis
- Frozen section
 - Not reasonable to section at 2 mm intervals
 - Destroys tissue – mets may be 'discarded' in the process of cutting the frozen section (?50%)
 - 'Freezing artifact' affects final interpretation
 - Time consuming for pathologist and surgeon

Intraoperative assessment Touch preps (cytology)

- Pathmanathan 2006: Sensitivity 31.1%
 - for macromets – 61.9%
 - For micromets and ITCs – 4.2%
- Pugliese 2006: 385 SLNs from breast
 - 48/65 macromets – 74%
 - 1/24 micromets – 4%
 - 0/36 ITCs – 0%

Intraoperative assessment Cytology vs. Frozen Section

- **Cytology Sensitivity:** Overall 40%
 - macromets – 78%
 - Micromets and ITCs – 9%
- **Frozen Section Sensitivity:** Overall 60%
 - macromets – 83%
 - micromets and ITCs – 20%

Menes et al *Ann Surg Oncol* 2003

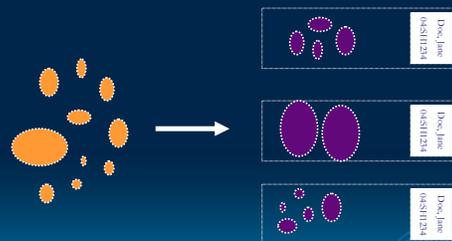
Intraoperative assessment

- Certainly useful if surgeon identifies a suspicious node intraoperatively
- Questionable utility for routine use
 - Time consuming for surgeon, lab personnel
 - Uses valuable OR time
 - Percentage of patients (especially micrometastatic) will require second surgery

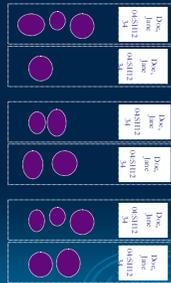
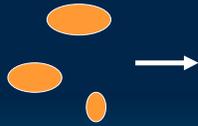
Impact on Pathology

Dramatic increase in workload

Handling a traditional lymph node dissection



The average sentinel node biopsy case



This results in a total of 24 H&E slides, 6 CAM5.2 IHC slides + control slides

MELANOMA case -- 4 nodes



Impact on Pathology-- What can be done?

- Coordination – long delays between injection and surgery result in higher numbers of 'hot' nodes
- Determine at outset the size of metastases that will have clinical impact – set protocol accordingly
- Consider the pathology department in the impact analysis carried out in the planning stage and budget accordingly

Summary

- Sentinel lymph node biopsy has benefits to the patient – morbidity
- Costly to pathology department – especially in pathologist and technologist time
- Detects metastases of unknown clinical significance – research required
- May miss clinically significant metastases
- Not practical to assess nodes intraoperatively
 - Highly false negative rate
 - Inefficient use of OR time and laboratory resources

