VALOR DE LA IMPRONTA EN GANGLIO CENTINELA CON EL SISTEMA OSNA

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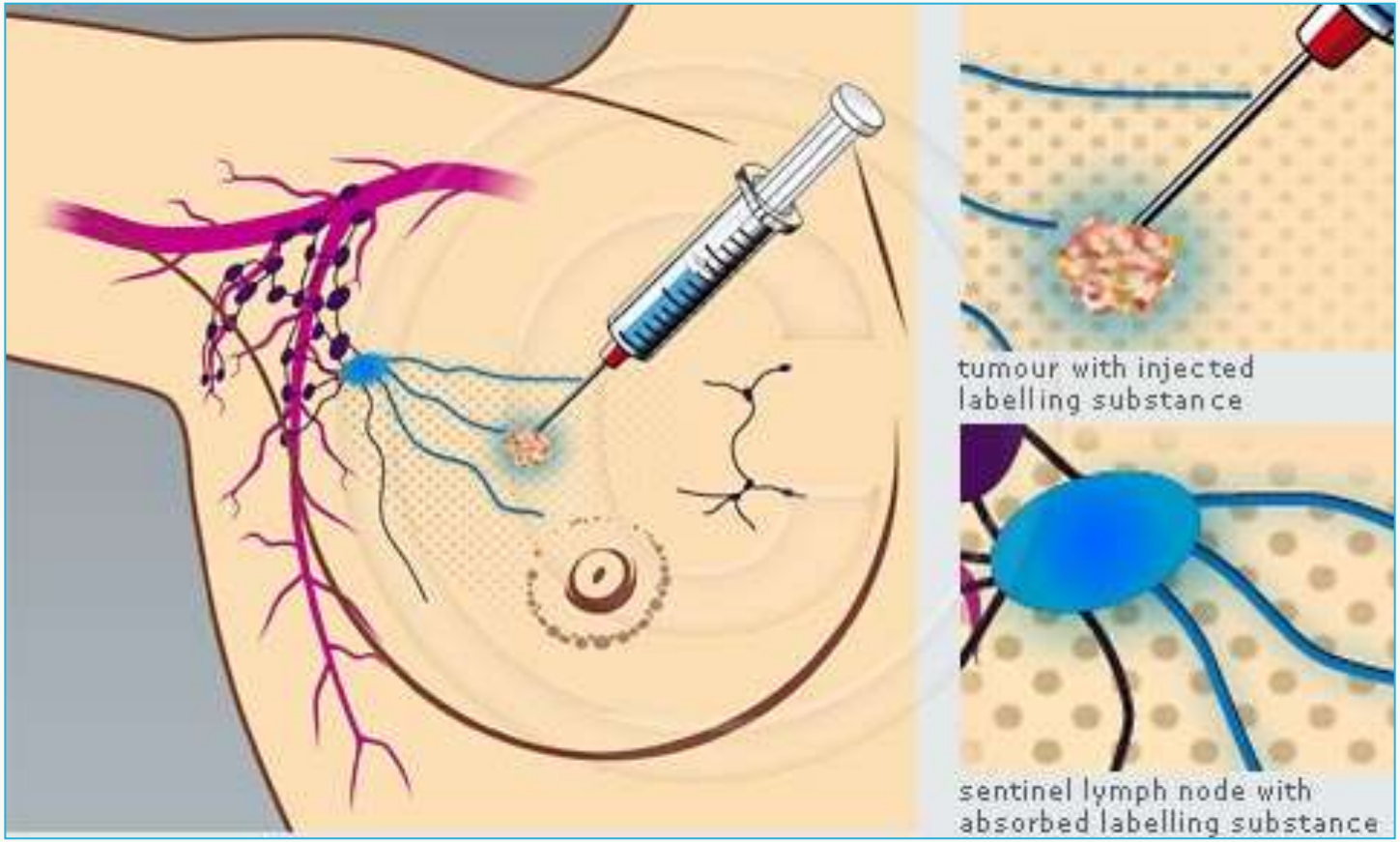
Utilidad de la impronta en el estudio del Ganglio Centinela con el método OSNA

- El estatus axilar es un factor pronóstico relevante, y marcador de la diseminación tumoral.
- El ganglio centinela es el primer ganglio afectado en la extensión linfática de un tumor.
- Morton, describe la técnica en melanoma (1992).
- Giuliano (1994-95): Improved axillary staging of breast cancer with sentinel lymphadenectomy.
- Causó una revolución en la comunidad científica porque el estudio de 1-4 ganglios permite estadificar la situación axilar y evitar la linfadenectomía.

Técnica del ganglio centinela



AJCC Sexta edición de TNM (2002)

- Tamaño de las metástasis en GC (Macro-Micro-ITC).
- Especificación de GC (sn).
- Se definen las ITC (“se detectan normalmente con IHC o métodos moleculares, pero se confirman con H-E”).
- Terminología:
  - pN0, pN0(i+), pN0(i-), pN0(mol+), pN0(mol-).

Método de estudio de ganglio centinela

- Impronta citológica

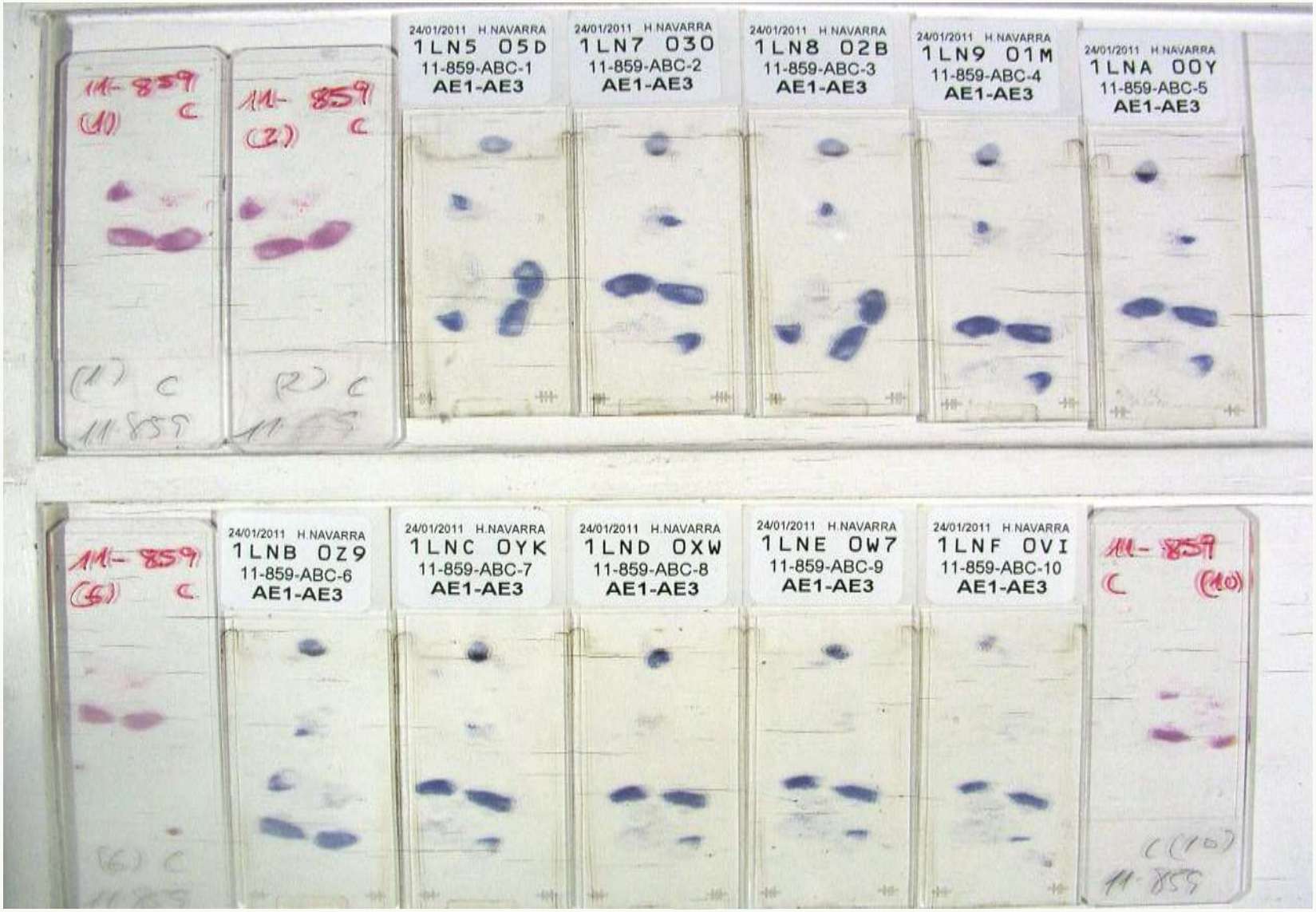
+

- Corte de congelación

Estudio diferido:

4 H-E + 10 queratina AE1/AE3

Método histológico



Utilidad de la impronta en el estudio del Ganglio Centinela con el método OSNA

Eur J Cancer. 2003 Aug;39(12):1654-67.

Pathological work-up of sentinel lymph nodes in breast cancer. Review of current data to be considered for the formulation of guidelines.

Cserni G, Amendoeira I, Apostolikas N, Bellocq JP, Bianchi S, Bussolati G, Boecker W, Borisch B, Connolly CE, Decker T, Dervan P, Drijkoningen M, Ellis IO, Elston CW, Eusebi V, Faverly D, Heikkila P, Holland R, Kerner H, Kulka J, Jacquemier J, Lacerda M, Martinez-Penuela J, De Miguel C, Peterse JL, Rank F, Regitnig P, Reiner A, Sapino A, Sigal-Zafrani B, Tanous AM, Thorstenson S, Zozaya E, Wells CA; European Working Group for Breast Screening Pathology.

Department of Pathology, Bács-Kiskun County Teaching Hospital, Kecskemét, Hungary.

Abstract

Controversies and inconsistencies regarding the pathological work-up of sentinel lymph nodes (SNs) led the European Working Group for Breast Screening Pathology (EWGBSP) to review published data and current evidence that can promote the formulation of European guidelines for the pathological work-up of SNs.

After an evaluation of the accuracy of SN biopsy as a staging procedure, the yields of different sectioning methods and the immunohistochemical detection of metastatic cells are reviewed.

Currently published data do not allow the significance of micrometastases or isolated tumour cells to be established, but it is suggested that approximately 18% of the cases may be associated with further nodal (non-SN) metastases, i.e. approximately 2% of all patients initially staged by SN biopsy.

The methods for the intraoperative and molecular assessment of SNs are also surveyed.

Efectividad intraoperatoria

Table 6
Studies on the intra-operative assessment of SNs

A	B	C	D	E	F	TP	TN	FP	FN	ACC (%)	SENS (%)	SPEC (%)	PPV (%)	NPV (%)	FNR (%)	FRR (%)
FS	[97]	28	1 (2)	HE	IHC	6	17	0	5	82	55	100	100	77	45	23
FS	[98]	47[a]	NI	HE	HE	10	36	0	1	98	91	100	100	97	9	3
FS	[99]	54	2	HE	Mult. HE + IHC	21	31	0	2	96	91	100	100	94	9	6
		74[a]	2	HE	Mult. HE + IHC	27	43	0	4	95	87	100	100	91	13	9
FS	[100]	62	⩾1	HE	HE + IHC same level	19	34	0	9	85	68	100	100	79	32	21
FS	[13]	96	3 (both sides)	HE	HE	24	68	0	4	96	86	100	100	94	14	6
FS	[101]	107	3 consec	HE	3 HE	32	57	0	18	83	64	100	100	76	36	24
FS	[102]	157	NI	HE	Mult. HE + IHC	41	116	0	18	90	69	100	100	87	31	13
FS	[103]	165[a]	NI	HE	Mult. HE at 2–3 mm	19	141	2	3	97	86	99	90	98	14	2
FS	[104]	203	2	HE	Mult. HE at 2 mm + IHC	53	132	1	17	91	76	99	98	89	24	11
IC + FS	[100]	38	⩾1	MG + IHC/HE	HE + IHC same level	3	25	0	10	92	77	100	100	89	23	11
IC + FS	[105]	278	1	DQ	HE same level	53	206	0	19	93	74	100	100	92	26	8
		278	1	DQ	Mult. HE + IHC	53	167	0	58	79	48	100	100	74	52	26
IC	[106]	25	1	RAL	NI	4	19	0	2	92	66	100	100	90	33	10
IC	[100]	38	1	MG + IHC	HE + IHC same level	6	25	0	7	82	46	100	100	78	54	22
IC	[99]	45	⩾2	DQ	Mult. HE + IHC	14	23	0	8	82	64	100	100	74	36	26
		59[a]	⩾2	DQ	Mult. HE + IHC	16	33	0	10	83	62	100	100	77	38	23
IC	[107]	55	= 2	HE	HE same level	14	40	0	1	98	93	100	100	98	7	2
IC	[108]	60	= 2	HE	Mult. HE + IHC	19	28	0	13	78	59	100	100	68	41	32
IC	[109]	65	⩾2 (1/slice)	P or DQ	Mult. HE + IHC	17	33	1[c]	14	77	55	97	94	70	45	30
IC	[110]	101	⩾2 (1/slice)	P	HE + IHC same level	30	67	1[c]	3	96	91	99	97	96	9	4
IC	[111]	109	2–6	Giemsa	Mult. HE + IHC	32	63	0	14	87	70	100	100	82	30	18
IC	[112]	124[a]	1	HE	HE same level	22	101	0	1	99	96	100	100	99	5	1
IC	[113]	148	= 2	Giemsa and P	3-level HE + IHC	40	86	2	20	85	67	98	95	81	33	19
IC	[114]	150	1	HE	3-level HE, IHC in some	20	113	0	17	89	54	100	100	87	46	13
IC	[115]	161[b]	2	IHC	Mult. HE + IHC	30	126	0	5	97	86	100	100	96	14	4
IC	[116]	381[b]	2	DQ	Mult. HE + IHC	15	254	1	35	88	30	100	94	88	70	12
IC	[103]	479[a]	>1	HE	Mult. HE at 2–3 mm	65	409	1	4	99	94	100	98	99	6	1

A, Method; B: reference; C: number of patients; D: number of levels studied intraoperatively; E: stains used intraoperatively; F: final histopathology details: SN, sentinel lymph node; TP, true positive cases; TN, true negative cases; FP, false-positive cases; FN, false-negative cases; ACC, accuracy; SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value; FNR, false-negative rate (FN/all node positives); FRR, false reassurance rate (FN/(FN + TN)); FS, frozen section; IC, imprint cytology; consec, consecutive; HE, haematoxylin + eosin; DQ, Diff-Quik; MG, May-Giemsa; P, Papanicolaou; RAL, rapid cytological stain RAL 555; IHC, immunohistochemistry for the demonstration of epithelial markers; Mult., multiple; NI, not indicated.

[a] On an SN (and not patient) basis.

[b] On a grossly negative SN (and not patient) basis.

[c] Cases reported as positive on intra-operative assessment and negative by final histology, that the authors did not interpret as a false-positive result, are presented here as false-positive findings, because the standard comparison in this table is the result of histology. Modified and updated from Ref. [95].

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QUALITY ASSURANCE GUIDELINES FOR PATHOLOGY, 2004

- **The chance of finding tumour in SNs is related to the proportion of tissue evaluated and the methods that are applied. A balance has to be struck between general applicability and optimal sensitivity**

Intraoperative examination:

- **Both frozen sections and imprint cytology have a risk of false negative classification, and false positive results have also been rarely reported. Any of these methods may be suitable for the intraoperative assessment of SNs, but none will adequately identify all metastases in these lymph nodes.**

- **Both methods have advantages and disadvantages and, in good hands, both methods have a similar sensitivity and specificity.**

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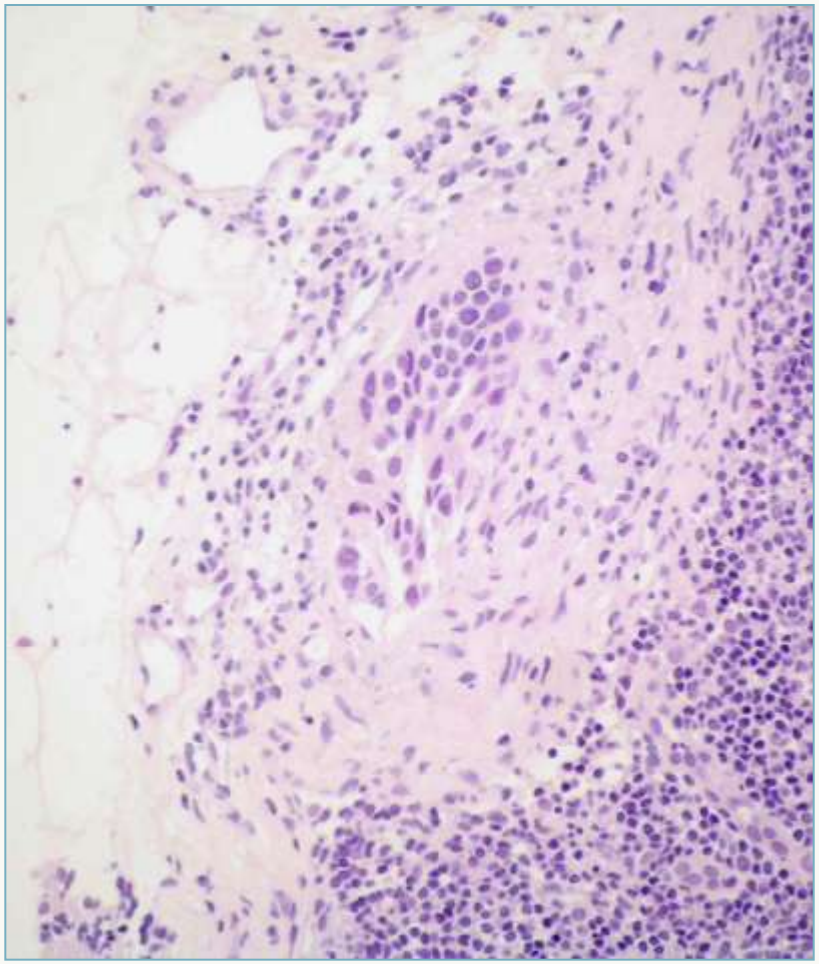
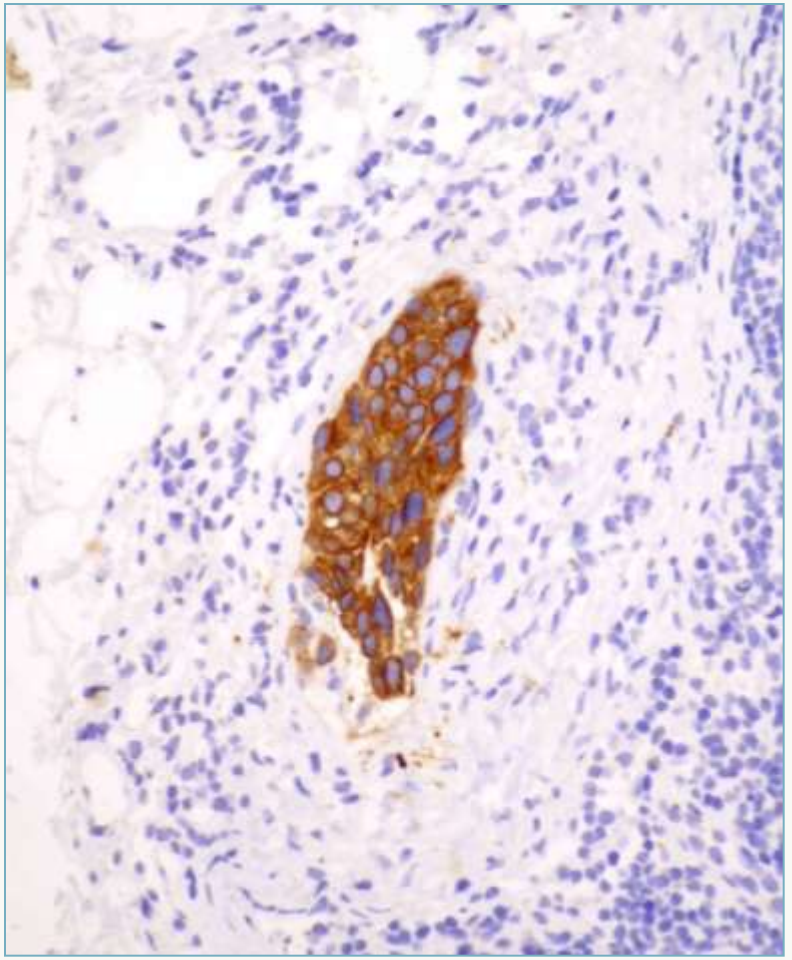
Impronta - Corte en congelación

Table 5
Advantages and disadvantages of intra-operative frozen section and imprint cytology assessment of SNs

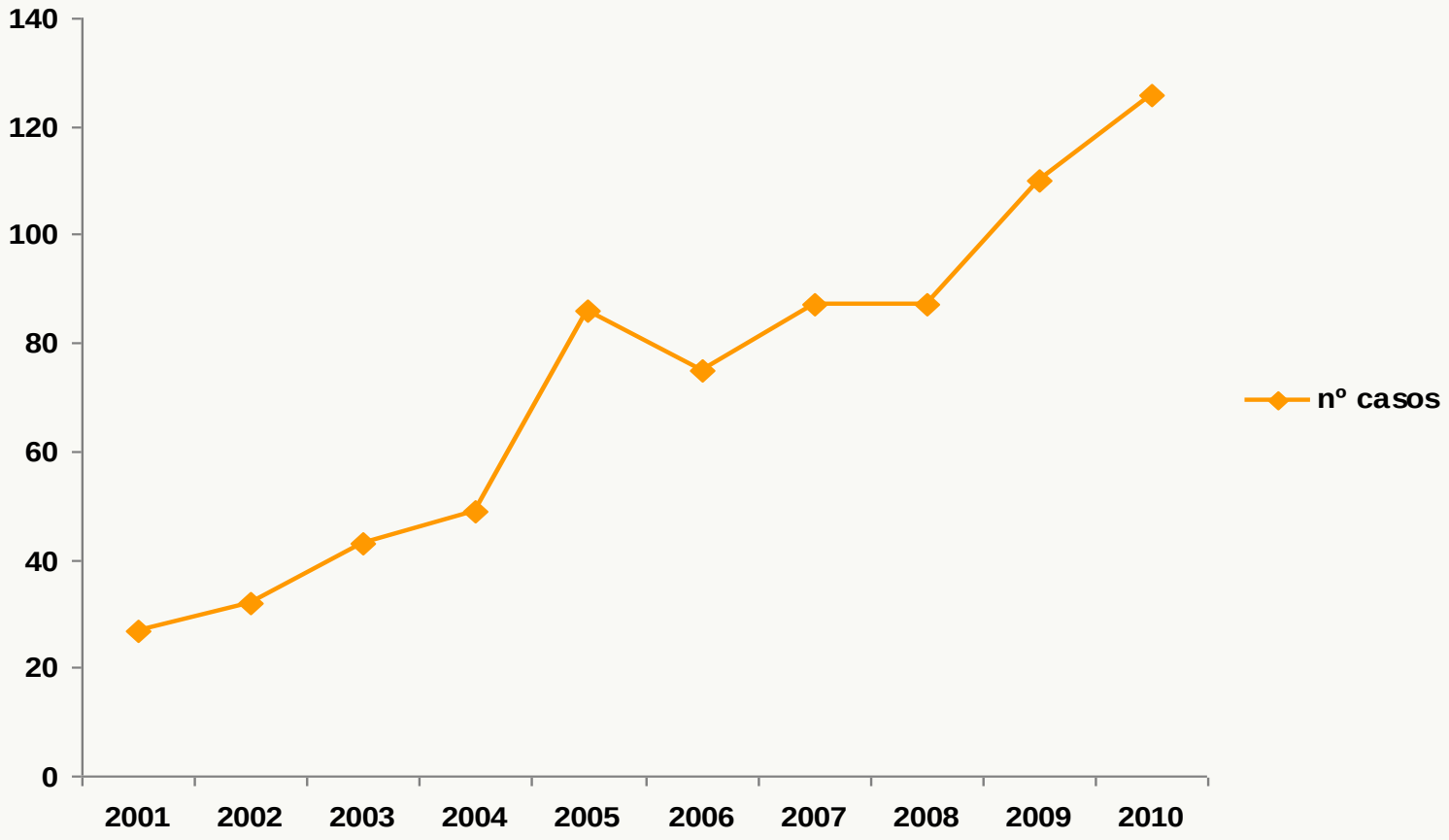
Advantages	Disadvantages
Frozen sections	
Tissue diagnosis (nodal architecture)	Freezing artifacts
Usually specific, less deferred diagnoses	Requires more time
Enables differentiation of macrometastases and micrometastases	Some tissue is lost
Histologists are more familiar with the method	More expensive
Can be complemented by rapid IHC	Sampling errors may occur
Imprint cytology	
Simple	Fewer cells assessed
Cheap	More indeterminate and deferred diagnoses
Rapid	Cannot differentiate between micrometastases and macrometastases
May give excellent cytological details	Sampling errors may occur
Requires cytology training	
Can be complemented by rapid IHC	

Tamaño de las metástasis





Evolución del ganglio centinela



Int. J. Cancer: **122,** 2562–2567 (2008)

Intra-operative rapid diagnostic method based on CK19 mRNA expression for the detection of lymph node metastases in breast cancer

Mike Visser[1], Mehdi Jiwa[2], Anja Horstman[2], Antoinette A.T.P. Brink[1], Rene P. Pol[1], Paul van Diest[3], Peter J.F. Snijders[1] and Chris J.L.M. Meijer[1]*

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Staging by sentinel node (SN) biopsy is the standard procedure for clinically node-negative breast cancer patients. Intra-operative analysis of the SN allows immediate axillary lymph node (ALN) dissection in SN positive patients, but a quick, reliable and reproducible method is lacking. We tested the suitability of a quantitative cytokeratin 19 (CK19) mRNA one step nucleic acid amplification (OSNA®) technique (OSNA-CK19) for intra-operative SN analysis. OSNA-CK19 involves a short manual sample preparation step and subsequent fully automated amplification of CK19 mRNA based on reverse transcription loop-mediated isothermal amplification, with results available within 30–40 min. OSNA-CK19 was compared to histological staining (Hematoxylin&Eosin and CAM5.2 and CK19 immunostaining) of 346 frozen ALNs from 32 breast cancer patients, using half of the lymph node for each method. 267 samples were negative and 61 positive by both methods. Three samples were histology positive and OSNA-CK19 negative. Fifteen samples were histology negative and OSNA-CK19 positive, 11 of which had copy numbers close to the cut-off level of OSNA-CK19. Seven of these 15 samples were RT-PCR positive for epithelial markers and/or showed CK19 protein expression by Western blot suggesting the presence of tumor deposits in the lymph node part investigated by OSNA-CK19. Concordance with histology was 94.8%, and 96.8% after exclusion of the latter 7 discordant cases. Sensitivity was 95.3% and specificity was 94.7% before and 97.1% after discordant case investigation. Our results indicate that OSNA-CK19 can potentially be useful in an intra-operative clinical setting to detect SN tumor involvement in breast cancer patients.

Molecular approaches such as real-time PCR have been applied for the detection of tumor deposits in lymph nodes of breast cancer patients and indicated higher sensitivity than histological investigations.[12–14] Results obtained with RT-PCR correlated with traditional predictors of prognosis[13]

CK19 mRNA is a suitable marker for identifying breast cancer deposits in lymph nodes because virtually all breast cancers express this cytoskeleton protein.[15] Recently, a new semi-automated molecular method for rapid intra-operative diagnosis of lymph node metastases in breast cancer patients has been developed using One step nucleic acid amplification (OSNA). The OSNA-CK19 assay (Sysmex, Kobe, Japan) is based on homogenisation of lymph node samples followed by real-time amplification and quantitation of cytokeratin 19 (CK19) mRNA directly from the lysate, with results available within 30 min for one SN and 40 min for 4 SNs. The quantitative molecular result is related to the size of the metastases.

In recent studies performed in Japan OSNA-CK19 has been found to be a potentially valuable intra-operative method for the detection of lymph node metastases in patients with gastric,[16] colorectal,[17] and breast cancer.[18] To find out whether this method has also potential to be a good intra-operative alternative for a more extensive postoperative histological work-up of SNs that is common in many European settings, we tested the performance of the OSNA-CK19 method in comparison with the standard histological method (staining step sections with H&E and pan cytokeratin staining) in 346 ALN from 32 Dutch breast cancer patients under-

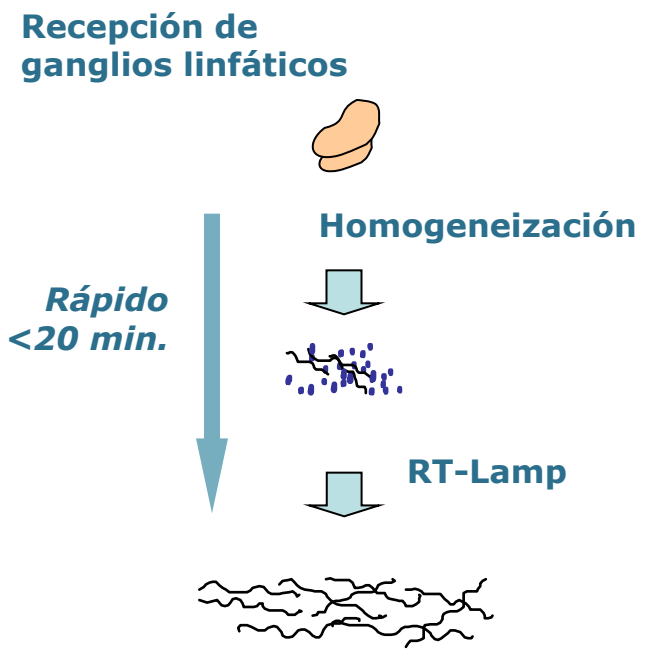
OSNA: one-step nucleic acid amplification method

- **RT-LAMP**: tecnología de replicación
- Amplificación isotérmica a **65ºC**.
- Duración de la amplificación **16'.**
- Más específico con **6 primers diferentes** (4 que le dan especificidad y 2 mayor velocidad).

- Puede observarse la **amplificación a tiempo real**.
- El equipo mide la **turbidez** de un subproducto (Pirofosfato de magnesio) de la replicación del ADN.
- **Risetime**: tiempo que tarda en producirse un valor de 0.1 de turbidez en la muestra. Se correlaciona con la cantidad de ARNm de CK19 que hay en la muestra y éste a su vez se correlaciona muy bien con el número de células tumorales.

OSNA: one-step nucleic acid amplification method

- One Step Nucleic Acid Amplification -
(OSNA)



- **¿Cuál es el marcador?**
  CK19.
- **¿Qué es lo que mide este marcador?**
  Medida semi-cuantitativa y cuantitativa del ARNm CK19.
- **¿Qué detecta?**
  Macrometástasis, Micrometástasis, ITC.
- **¿Puede analizar más de un ganglio?**
  Hasta 4 a la vez.
- **¿Se identifican las muestras?**
  Sí, evitando errores de muestreo.

OSNA: one-step nucleic acid amplification method

Resultados:

1. Confirmar el Risetime del Control Negativo que sea ND

2. Confirmar el Risetime de PC que sea 11 + / - 2

Muestra no diluida CK19		**Resultado**	
	5000 ≤ copias	(++)	
	250 ≤ copias < 5000	(+)	
	100 ≤ copias < 250	**(-)L**	(+) I Sólo si la muestra diluida a 1:10 tiene más de 250 ejemplares
	copias < 100	(-)	

Flag debido a la inhibición: Los resultados se muestran como (+) I.
Baja expresión se mostrarán como (-) L (no es relevante!)

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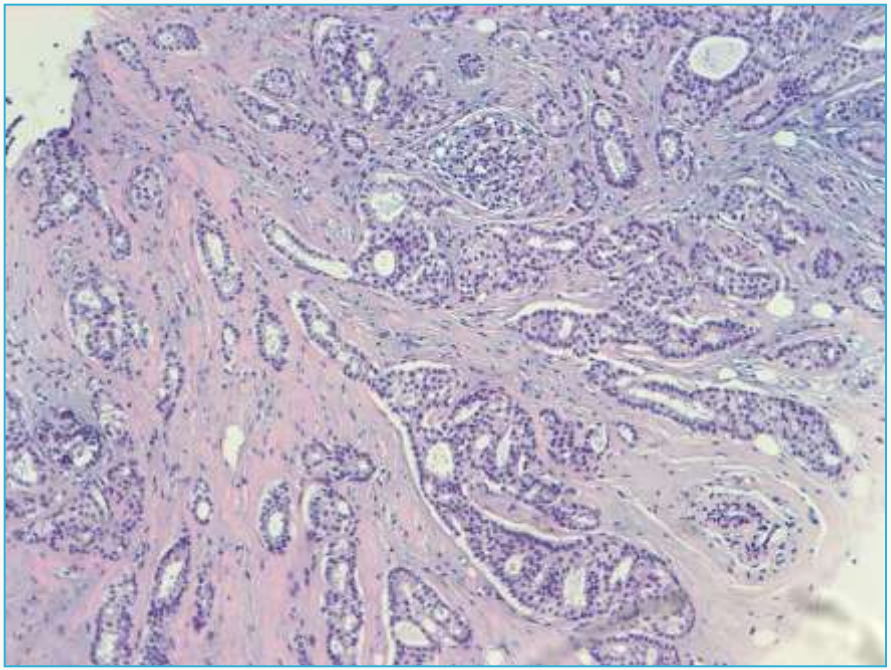
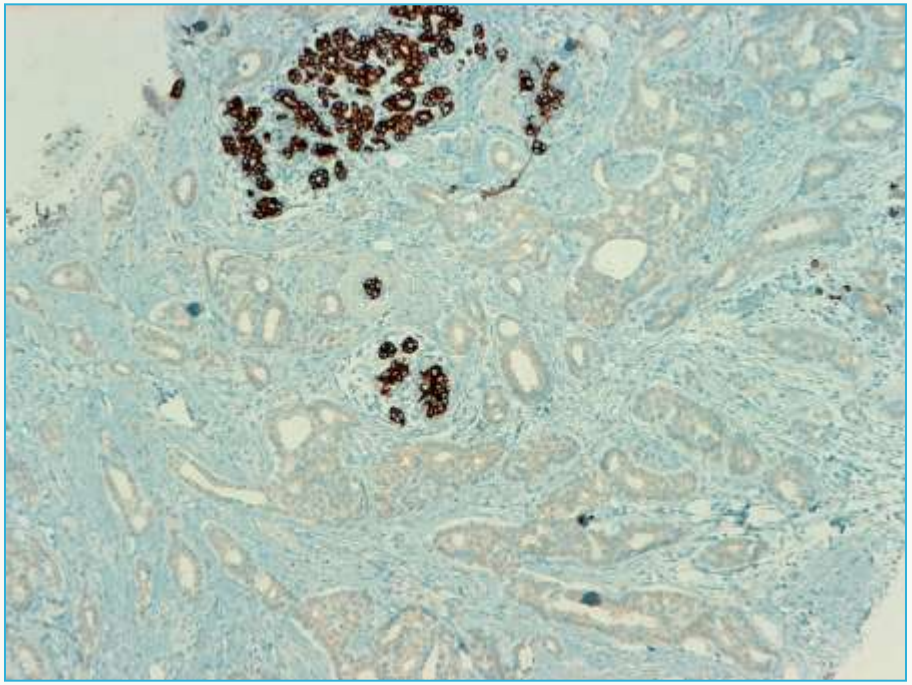
Queratina 19 negativo





STUDY ABOUT (PRESENCE OF METASTASIS) IN SENTINEL LYMPH NODE BY ONE-STEP NUCLEIC ACID AMPLIFICATION (OSNA) ASSAY

A Córdoba, F Vicente, D Guerrero, M Mercado, I Rodriguez, ML Gomez, JM Martinez-Peñuela, I Eguaras.
Department of Pathology, Hospital of Navarra, Navarra Health Service
Biomedical Research Center, Navarra Health Service
Department of Surgery, Hospital of Navarra, Navarra Health Service

Results

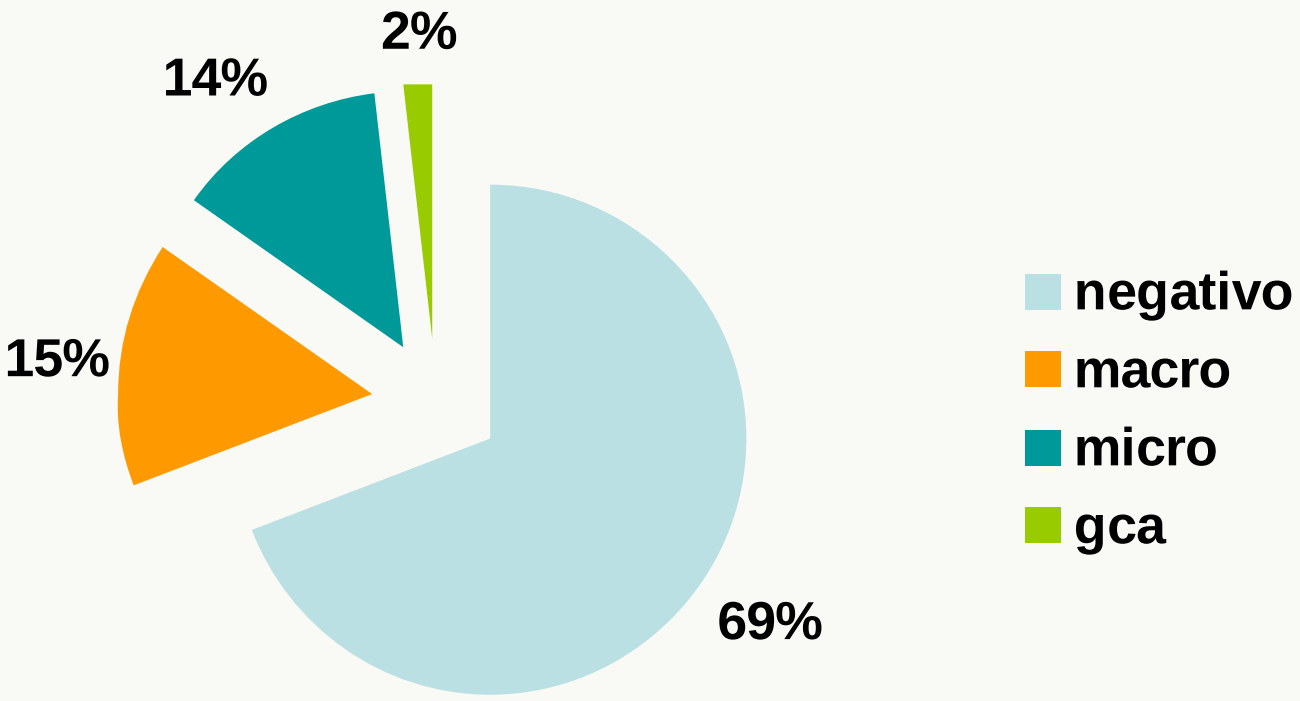
- We found 11 patients (17%) with metastases in the SLNs analysed by the conventional method, and 4 out of these were found in preoperative (6.25%) by frozen section and 7 (10.9%) in the subsequent conventional study (H&E, CK). Hence, these 7 patients were false-negative cases.
  - Among the cases detected in the subsequent study 4 were micrometastasis and 3 were macrometastasis.
- The OSNA assay detected 17 (29.8%) metastasis.
  - The distribution according to the size of the metastasis of these metastasis was 9 micrometastasis and 8 macrometastasis.

Material and methods

- Comparative study of the presence of metastasis in SLNs was performed by frozen section (64 patients, 131 lymph nodes) and OSNA assay (57 patients, 114 lymph nodes) for a period of 6 months each at the Hospital de Navarra.
- The conventional study after the preoperative study was performed in 4 sections stained with H&E and 10 sections stained with keratin AE1-AE3.

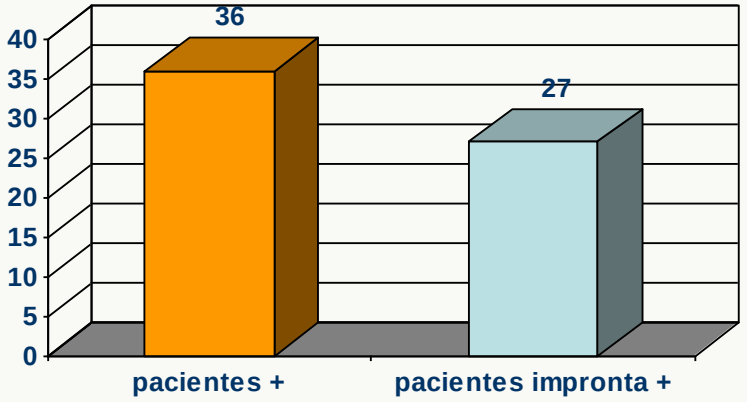
Resultados globales 2010

- Número de pacientes estudiados: 123.
- Número total de ganglios estudiados:239
- Número de pacientes positivos: 36.

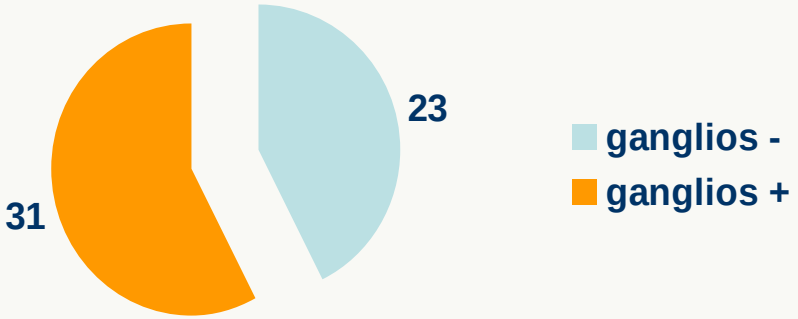


Impronta resultados:

- Número de pacientes positivos + impronta: 27
- Pacientes con ganglio +: 36

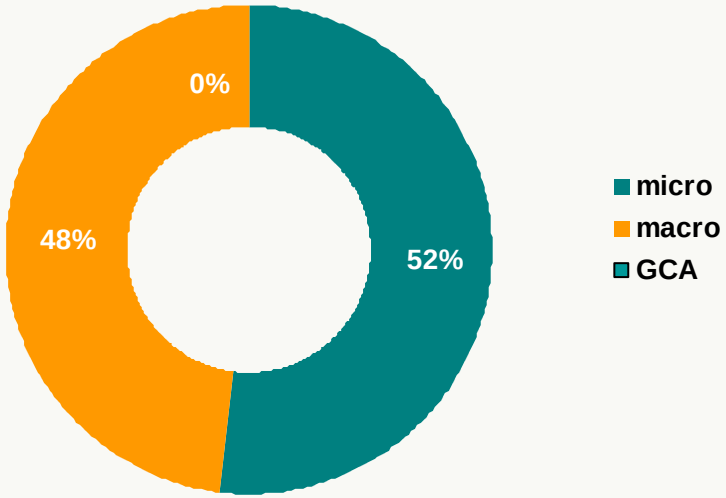


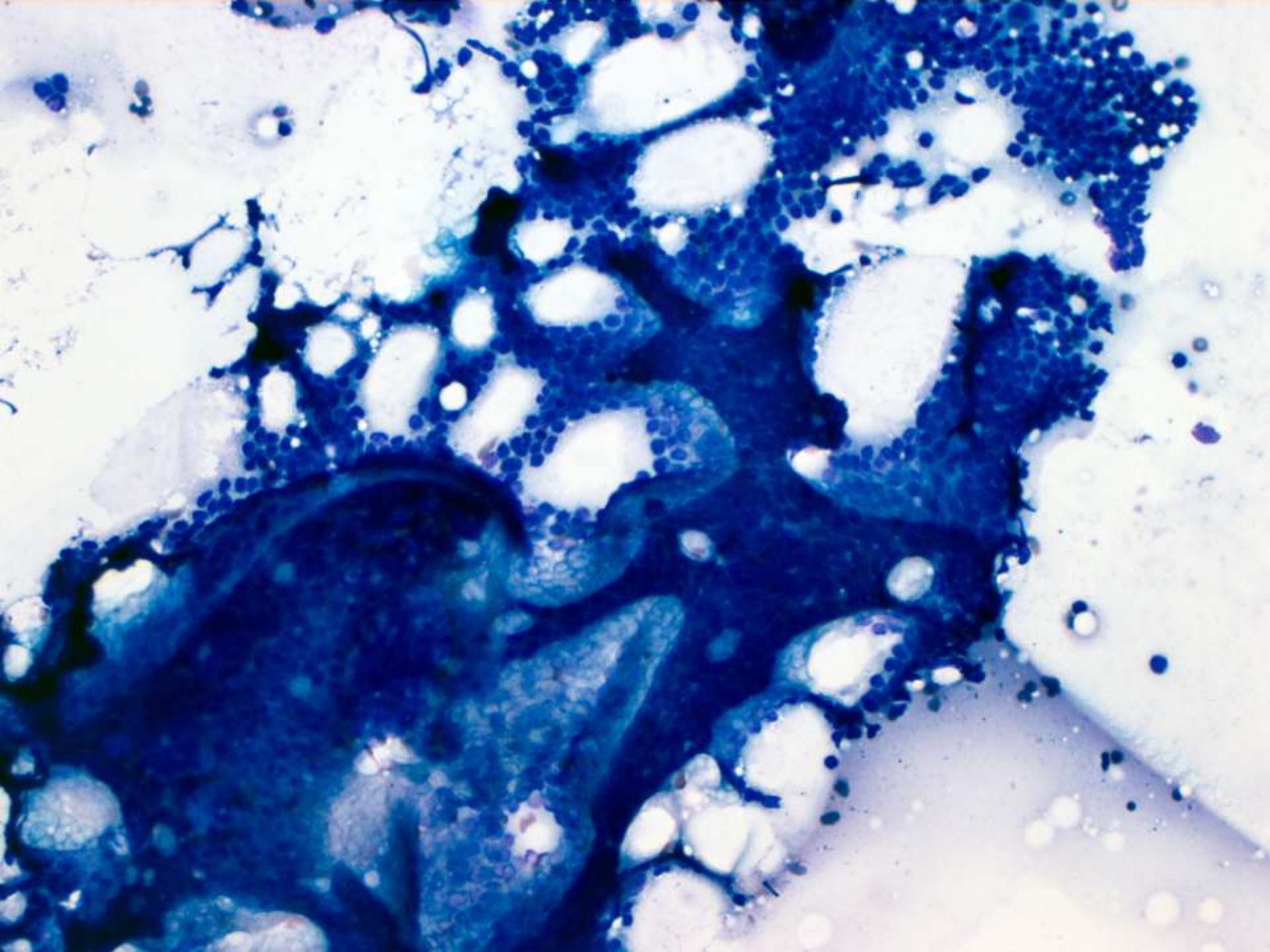
- Número de ganglios estudiados: 54.
- Número de ganglios positivos con OSNA: 31.

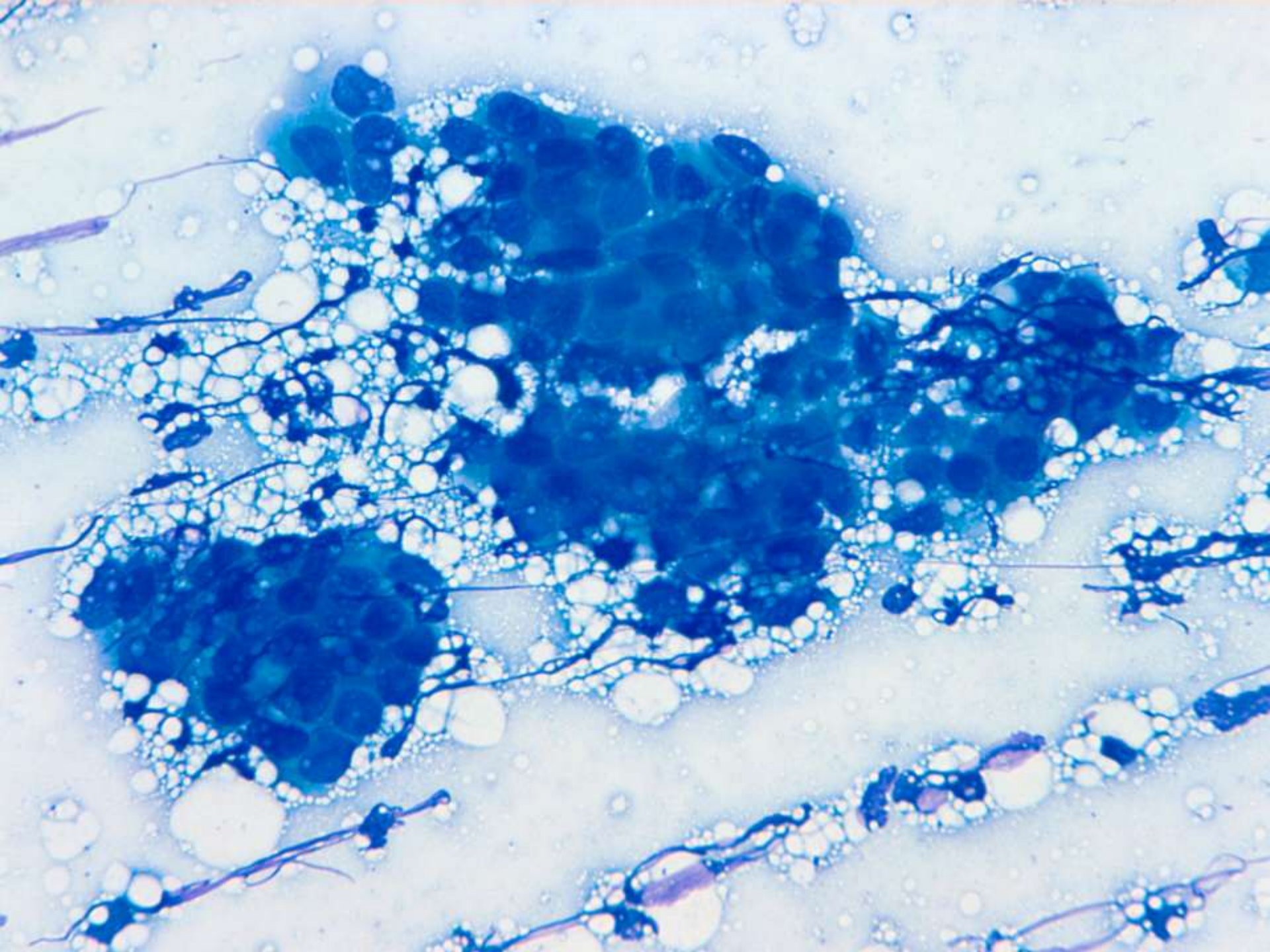


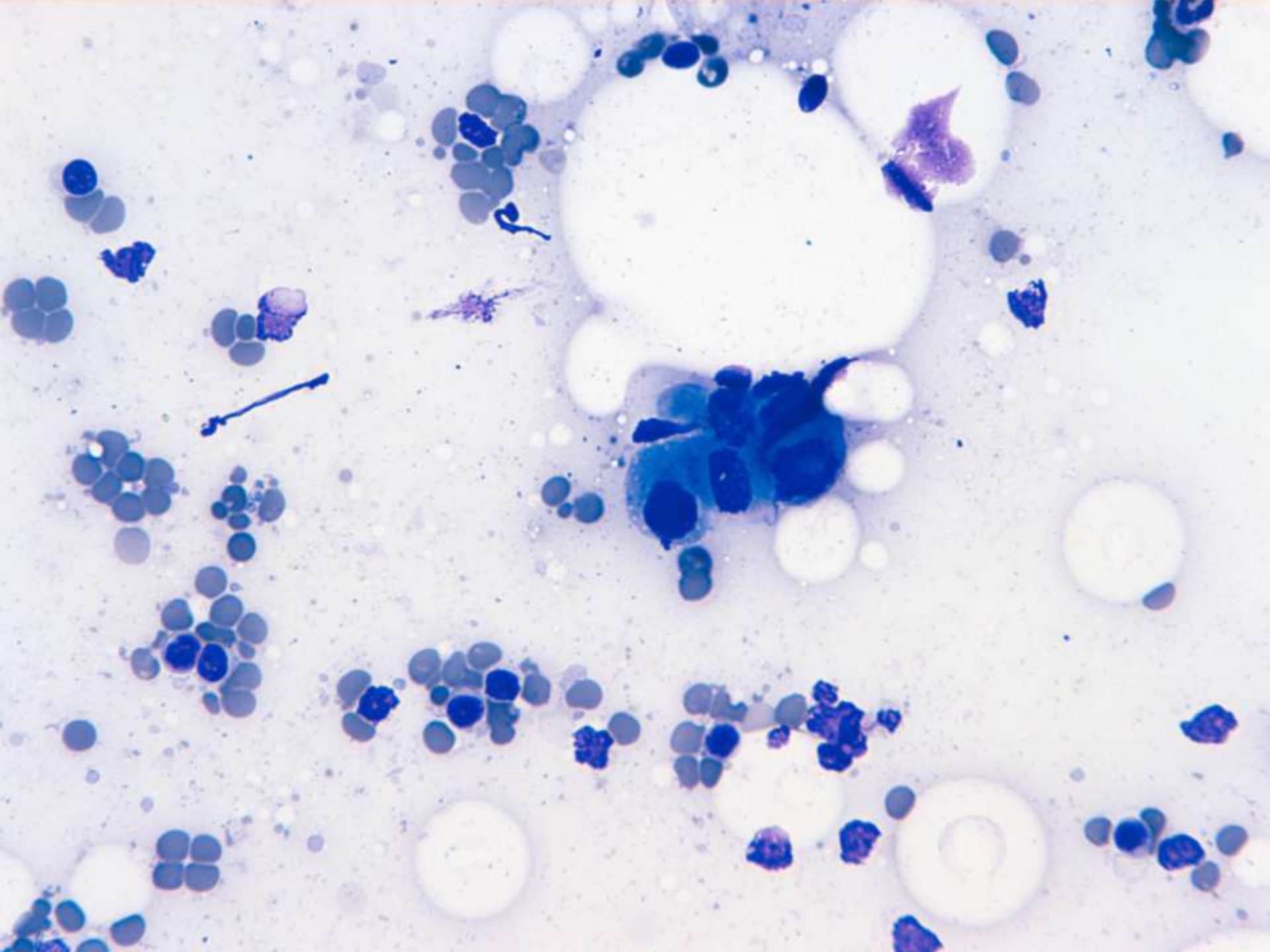
Tamaño de las metástasis

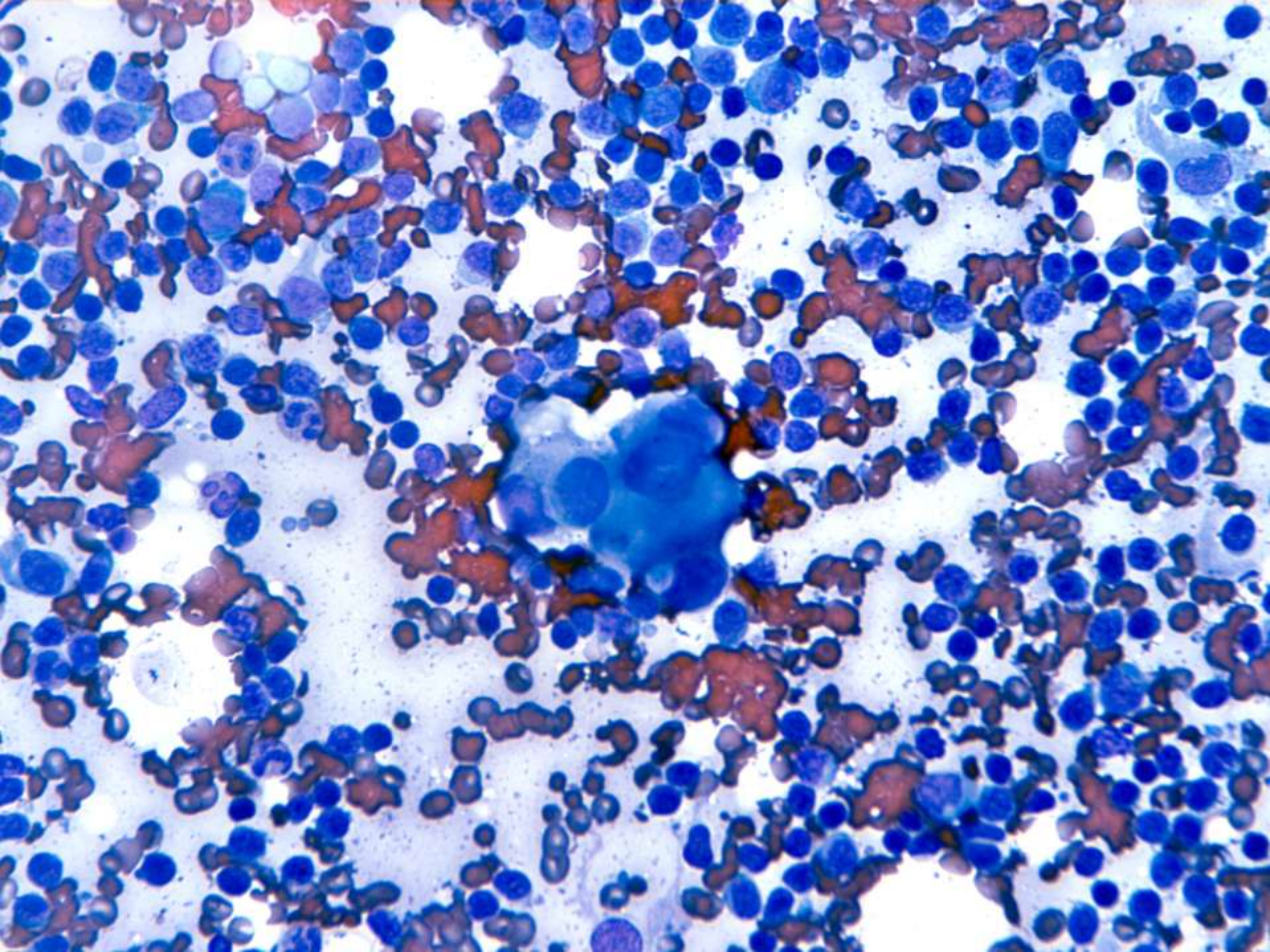
- Macrometástasis: 13 (42%).
- Micrometástasis: 18 (58%).
- Grupo celular aislado: 0.





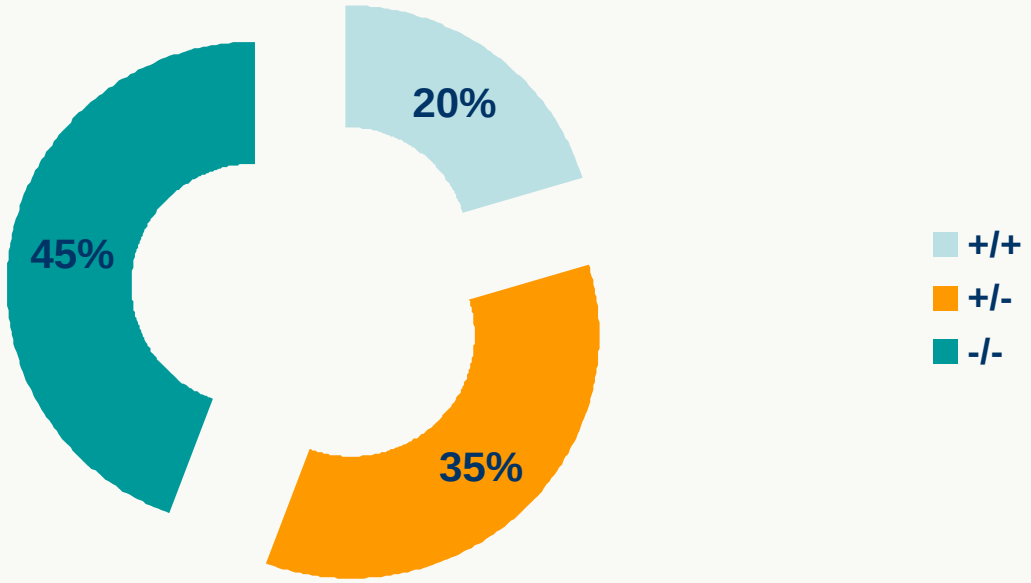






Impronta resultados:

- Ganglios OSNA+/impronta+: 11(20,3%).
- Ganglios OSNA+/impronta-:19(35,1%).
- Ganglios OSNA-/impronta-: 24(44,4%).



Sensibilidad: 36%

Especificidad:100%

VPN: 0,72%

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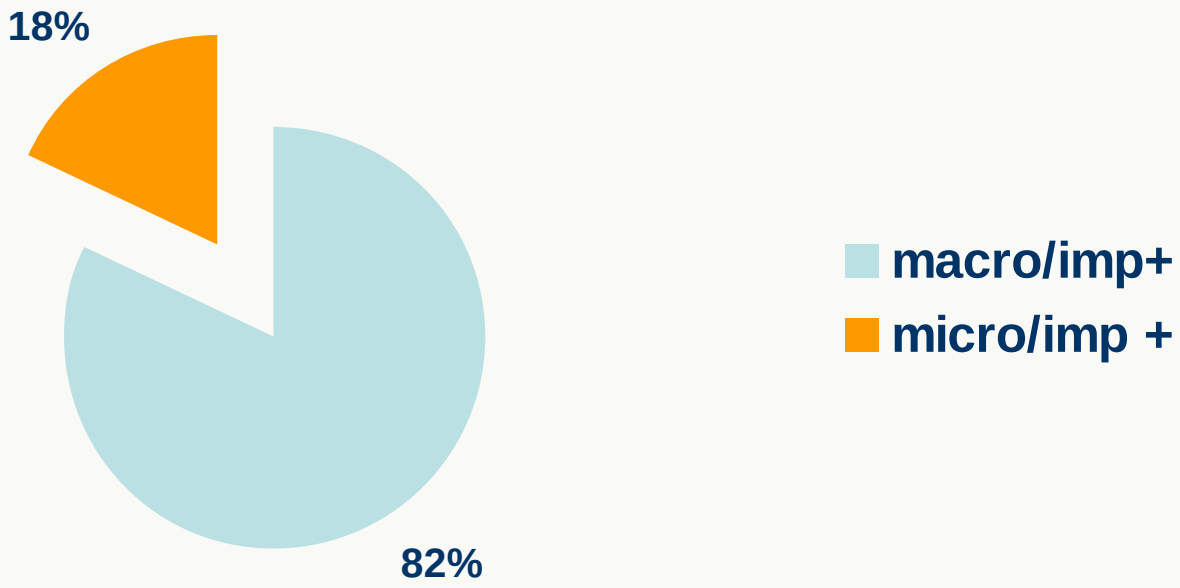
Impronta resultados:

- Macro OSNA / impronta+: 9 (81,8%)
- Micro OSNA / impronta+:2 (18,1%)
- GCA OSNA / impronta+: 0 (0%)



Impronta resultados:

- Impronta +/número de copias CK19: 2x10.6
- Impronta -/número de copias CK19: 400

UPDATE to the GUIDELINES (EWGBSP)

Thus, should the first approach of using the whole SLN for molecular assay be favoured, **it is recommended that at least a frozen section or a (touch or scrape) cytology specimen is taken**, examined and archived by pathologists for microscopic evaluation of the SLN tissue

Consenso de Valencia 2010

0214-1582/2010/23/5/201-208
Revista de Senología y Patología Mamaria

Rev Senología Patol Mam
2010; 23 (5), pp. 201-208

ARTÍCULO ESPECIAL

Actualización del consenso sobre la biopsia selectiva del ganglio centinela en el cáncer de mama

Sociedad Española de Senología y Patología Mamaria*

- Se recomienda utilizar el ganglio en su totalidad. Es aceptable realizar una citología por impronta antes de homogeneizar el ganglio para el estudio molecular, tratando de evitar cualquier causa de contaminación.

Conclusiones

- Confirmar la presencia de tumor.
- Conservar muestra citológica de dicho tumor.
- Importancia añadida en los casos que se someten a neoadyuvancia y se puede producir una regresión histológica completa.
- Seguir las recomendaciones del Grupo EWGBSP.
- No interfiere en las ventajas que ofrece el método de estudio completo y definitivo del ganglio en el estudio intraoperatorio.

DATOS CLÍNICOS ACUMULADOS	inicio	07-10-09
	final	05-01-11

Número de pacientes	146
Número de ganglios	273
Número de muestras	327
Media ganglios/paciente	1,9

Pacientes negativos (-)	103	71%
Pacientes positivos	40	27%
Pacientes con macrometástasis (++)	21	14%
Pacientes con micrometástasis (+)	19	13%
Pacientes con baja expresión (-)L	3	2%