

OSNA – a new method for lymph node staging in colorectal carcinoma

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Background:

Reliable staging of colorectal carcinomas (CRC) depends on the histopathological examination of the surgically removed specimen. Accurate evaluation for lymphatic metastases which is a main prognostic indicator correlates with the number of the investigated lymph nodes. Extensive conventional serial sections and staining techniques should be replaced by high throughput methods allowing correct lymph node staging in adequate time.

Material and Methods:

This study included 191 lymph nodes (LN) from 191 patients with the diagnosis of CRC subjected to inpatient treatment at the Department of Surgery, University of Erlangen-Nuremberg (Germany) between 2001 – 2003. LN were cut into 4 equal slices (a, b, c, d), each of 1 or 2 mm, or into halves if the LN was too small for this slicing procedure. Slices a and c were analysed by One Step Nucleic Acid Amplification (OSNA), slices b and d by histology (figure 1). 5 levels of histology for each slice (b, d) were performed. Each level consisted of 2 sections, one was stained with Haematoxylin & Eosin (H&E) and the other one was used for CK19 immunohistochemistry (IHC) (figure 2). The final histological result was based on H&E staining. For the OSNA assay, the slices a and c were shortly homogenised and centrifuged. Afterwards cytokeratin 19 (CK19) mRNA was automatically amplified in the RD-100i (Sysmex, Norderstedt, Germany) directly from the sample homogenate with no RNA purification necessary (figure 3). The quantitative result is released after a total of 30-40 minutes for 3-4 lymph nodes. OSNA results were categorised into -, +, ++, and also quantitatively described by CK19 mRNA copy number.

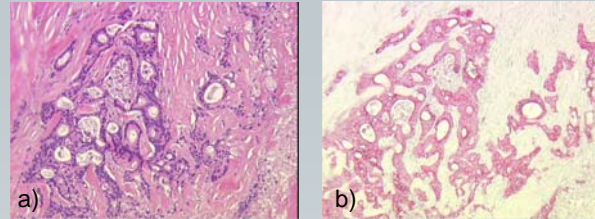


Figure 2: a) H&E staining of CRC lymph node metastasis, b) IHC CK19 positive lymph node metastasis

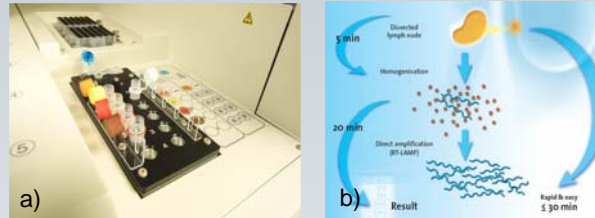


Figure 3: a) OSNA reagent and pipette tip unit of the OSNA instrument (Ready-to-use reagent kit, real-time amplification of CK19 mRNA), b) In the OSNA assay the mRNA is directly amplified from the lysate of the lymph node, with results available after 30-40 minutes

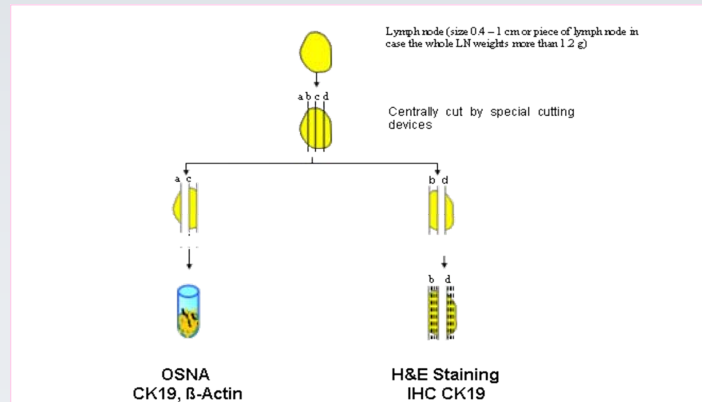


Figure 1: Experimental procedure

No.	Histology		OSNA			
	Size	Description	Copy number	Original run +/-	Second run +/-	Copy number +/-
1	8 mm	Macrometastasis, only in slice b, not d	< 250	-	4100	+
2	5 mm	Macrometastasis, calcified tissue	< 250	-	Not done	
3	2 mm	Macrometastasis, only in slice b, only in level 1 with H&E, not IHC	< 250	-	Not done	
4	3 mm	Only level 2	< 250	-	Not done	
5	3 mm	Only in initial level with H&E, not IHC	< 250	-	Not done	
6	-		540	+	Not done	
7	-		1300	+	Not done	
8	-		1300	+	Not done	
9	-		2700	+	< 250	-
10	-	Positive with IHC in level 1	25000	++	Not done	

Table 1: Description of discordant cases.

Results:

143 samples gave a negative result and 38 samples gave a positive result with both methods (table 2). Isolated tumour cells were not found. 5 samples were histology +/OSNA -, and 5 samples were histology -/OSNA + (table 2).

Out of the 5 histology +/OSNA - samples, sample no. 1 (table 1) contained a macrometastasis, but only in one of the two slices used for histology. Discordant case No. 2 contained a 5 mm macrometastasis. The OSNA result for CK19 was negative and also the beta-actin value was right on the cut-off level (not shown), suggesting that the amount of RNA contained in the homogenate might have been insufficient for CK19 mRNA amplification. Samples no. 3 - 5 contained metastases confined to one of the five levels, indicative of tissue allocation bias, meaning that tumour deposits are restricted to the slice or slices either used for OSNA or histology. The same might apply for samples 6-8. Sample no. 9 was positive in the first OSNA run but negative upon repetition so a sample mix-up cannot be excluded. Lymph node no. 10 exhibited very high CK19 mRNA copy number, and IHC gave positive results in level 1.

In summary, the concordance rate was 94.8%, the sensitivity 88.4%, and the specificity 96.6%. However, it cannot be excluded that some of these discordant results are due to TAB.

OSNA	Histological investigation		
	Macrometastases	Micrometastases	Negative
++	28	2	1
+	8	-	4
-	5	-	143
Total	41	2	148

Table 2: OSNA in comparison to histological investigation.

Conclusion:

OSNA is a new and reliable method for molecular staging of lymphatic metastasis in CRC. It can be applied as a rapid diagnostic tool to estimate tumour involvement of lymph nodes during staging of CRC.