



**HAL**  
open science

## actors affecting false-negative rates on ex vivo sentinel lymph node mapping in colorectal cancer

A. Sommariva, P.M. Donisi, B. Gnocato, R. Vianello, V. Stracca Pansa, G. Zaninotto

### ► To cite this version:

A. Sommariva, P.M. Donisi, B. Gnocato, R. Vianello, V. Stracca Pansa, et al.. actors affecting false-negative rates on ex vivo sentinel lymph node mapping in colorectal cancer. *EJSO - European Journal of Surgical Oncology*, 2010, 36 (2), pp.130. 10.1016/j.ejso.2009.06.007 . hal-00557761

**HAL Id: hal-00557761**

**<https://hal.science/hal-00557761>**

Submitted on 20 Jan 2011

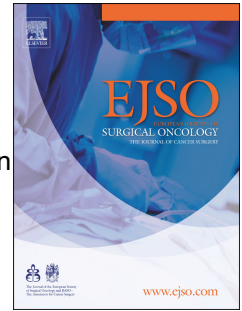
**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Accepted Manuscript

Title: Factors affecting false-negative rates on ex vivo sentinel lymph node mapping in colorectal cancer

Authors: A. Sommariva, P.M. Donisi, B. Gnocato, R. Vianello, V. Stracca Pansa, G. Zaninotto



PII: S0748-7983(09)00199-1

DOI: [10.1016/j.ejso.2009.06.007](https://doi.org/10.1016/j.ejso.2009.06.007)

Reference: YEJSO 2851

To appear in: *European Journal of Surgical Oncology*

Accepted Date: 15 June 2009

Please cite this article as: Sommariva A, Donisi PM, Gnocato B, Vianello R, Pansa VS, Zaninotto G. Factors affecting false-negative rates on ex vivo sentinel lymph node mapping in colorectal cancer, *European Journal of Surgical Oncology* (2009), doi: 10.1016/j.ejso.2009.06.007

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

FACTORS AFFECTING FALSE-NEGATIVE RATES ON EX VIVO SENTINEL LYMPH NODE MAPPING IN  
COLORECTAL CANCER

A. Sommariva M.D.<sup>1</sup>, P.M. Donisi M.D.<sup>2</sup>, B. Gnocco M.D.<sup>1</sup>, R. Vianello M.D.<sup>2</sup>, V. Stracca Pansa  
M.D.<sup>2</sup>, G. Zaninotto M.D.<sup>1</sup>

<sup>1</sup>Department of Surgery, Santi Giovanni e Paolo Hospital, Castello 6777, Venice, Italy

<sup>2</sup>Department of Pathology, Santi Giovanni e Paolo Hospital, Castello 6777, Venice, Italy

Oral presentation at the 13<sup>th</sup> Congress of the European Society of Surgical Oncology, Venice, Italy,  
November 30 - December 2, 2006

**Word count:**

text: 1832 words

abstract: 247 words

**References:** 22

**Abstract**

**PURPOSE:** Despite the increasing use of sentinel lymph node (SLN) mapping after colorectal cancer resection, reported node identification and false-negative rates vary considerably. The main aim of this prospective study was to quantify the false-negative rates on SLN mapping after resection and to evaluate factors influencing them.

**METHODS:** Sixty-nine patients with biopsy-proven cancer of the colon and rectum underwent SLN mapping according to a protocol involving the *ex vivo* submucosal and peritumoral injection of 2-4 ml of Patent Blue V dye. All lymph nodes visualized were marked as SLN and totally embedded, then two 4  $\mu$ m sections were cut for hematoxylin and eosin staining, and cytokeratin (AE1/AE3) immunostaining. A standard examination of the whole specimen and of the regional non-sentinel lymph nodes was also performed.

**RESULTS:** SLNs were identified in 97.3% of the evaluable cases. A mean 5.0 SLNs were removed per patient (SD  $\pm$ 4.2). Nine false negatives were identified. Rectal cancer, tumor size > 60 mm, number of metastatic non-sentinel lymph nodes, and mucinous tumors were associated with false-negative SLNs. At multivariate analysis, a rectal location and mucinous differentiation were independently associated with false-negative SLNs.

**CONCLUSIONS:** *Ex-vivo* SLN mapping after colorectal cancer surgery is technically feasible with a high identification rate. Tumor size and stage, rectal involvement and a mucinous histology seem to interfere with the reliability of SLN staging. It is mandatory to standardize the procedure and selection criteria in order to deal with the question of the reliability of SLN mapping in colorectal cancer.

## Introduction

Lymph node metastases are the most important predictors of survival in patients with potentially curable colorectal cancer (CRC). Adjuvant chemotherapy is given only to patients with positive nodes, whereas node-negative patients are considered cured by surgery [1,2]. Up to 30% of patients with negative nodes will recur, however, in the course of their lives [3]. This is at least partially due to understaging of the regional lymph nodes: patients with metastatic nodal disease that goes unnoticed are not given adjuvant chemotherapy and are at higher risk of recurrence.

There are two possible reasons why regional nodes go understaged:

1. not all metastatic nodes are identified due to an inadequate extension of the surgical resection, or some are overlooked during standard pathological dissection; 70% of metastatic lymph nodes are <5 mm in size and may escape notice because of their small size [4]. Cawthorn et al [5] recommended using a xylene alcohol clearance technique to increase the number of nodes identified, but the procedure is time-consuming and has not been widely adopted;
2. some metastatic deposits in harvested nodes are too small to be detected in 1-2 sections stained with hematoxylin and eosin (H&E) at standard pathological analysis. Stepwise sectioning of the nodes and the extensive use of immunohistochemistry and molecular analysis (reverse transcription polymerase chain reaction, RT-PCR) on the lymph nodes may improve the detection threshold for such metastases but, here again, such methods are labor-intensive and expensive, and they cannot be used routinely on all lymph nodes detected [6].

Sentinel lymph node mapping (SLNM) has been recommended in cases of CRC to help overcome these problems and improve disease staging [7-9]. Unlike the situation in breast cancer and melanoma, where the SLN concept has been used to lower the morbidity of regional lymphadenectomy [10,11], SLNM for CRC enables the pathologist to focus on the node(s) at

greatest risk of spread and examine these nodes with multiple slices and immunohistochemistry, and/or RT-PCR, and thereby detect metastatic tumor cells that would otherwise go unrecognized.

The results achieved with SLNM in CRC vary, however, in terms of the identification, false-negative and upstaging rates [12,13] and several questions remain to be addressed. It is still not clear which patients benefit most from this procedure (case selection), the best timing of the injection, the best tracer, the ultrastaging protocol and the prognostic value of micrometastatic cells detected in SLN.

The aim of the present study was to quantify the detection and false-negative rates achieved by *ex vivo* sentinel node mapping in CRC, and to assess the factors influencing these rates.

## Patients and Methods

### *Patient selection*

All patients were prospectively selected for SLNM according to a preoperative and intra-operative staging protocol, if they met the following criteria:

1. biopsy-confirmed colorectal cancer
2. no evidence of metastatic disease at preoperative staging (chest X-ray, liver ultrasound or abdominal CT scan)
3. no advanced rectal tumor (pelvic MRI)
4. elective surgery
5. no prior colorectal resection
6. no (previously undetected) liver metastases found at surgery
7. no peritoneal carcinosis or locally-advanced disease invading adjacent organs.

All patients gave their written, informed consent. The standard surgical procedure included: high division of the mesenteric vessels (for colon cancer), or division at the origin of the inferior

mesenteric vessels and total mesorectal excision (for rectal malignancies). Every suspect lymph node outside the resection field was retrieved and prepared for frozen section and pathological examination. Tumors were staged according to the AJCC/TNM [14]. The tumor's site, macroscopic features, maximal size, T and N stages, grade, mucinous features, and lymphatic or venous invasion were also recorded.

#### *Sentinel lymph node mapping procedure and pathological analysis*

Dye was injected by two surgeons (AS and BG) within 15 minutes of resecting the specimen, which was opened along the antimesenteric (or antimesorectal) border and 1-4 ml of Patent Blue V dye in 2.5% solution (Monico SpA, Mestre, Italy) were injected into the submucosa along the edge of the tumor. Sufficient dye was injected to color the submucosal layer around the tumor. No massage was performed. A few minutes later, the pericolic and perirectal tissue was systematically searched and all blue lymph nodes were retrieved and classified as SLNs. For rectal cases, the specimen was incised longitudinally along the antimesenteric border for posterior tumors and along the lateral edge for anterior tumors. The mesorectum was opened, paying attention to maintain the anatomical relationship between rectum, tumor and mesorectal fascia. A standard examination of the whole specimen, and of the regional non-sentinel lymph nodes (NSLNs) was subsequently performed. The SLNs were embedded in paraffin and two consecutive 4  $\mu$ m thick tissue sections were cut on two levels 200  $\mu$ m apart. The sections were stained with H&E and immunostained with mononuclear antibody for cytokeratin (Keratin AE1/AE3, Pan Ab-1 NeoMarkers, Inc Fremont CA, USA). Standard H&E-stained sections (2 per node) were obtained from the NSLNs. Micrometastases and isolated tumor cells were defined according to the guidelines of the International Union Against Cancer [15].

### *Statistical analysis*

The identification rate (IR) was defined as the number of mapping procedures finding at least one blue node out of the total number of mapping procedures performed. False negative cases (FN) were defined as those where the SLNs were negative but the NSLNs were positive. True positive cases (TP) were considered as those where the SLNs were positive and the NSLNs were positive or negative. The false-negative rate (FNR) was calculated using the formula  $(FN/FN+TP)$ . Continuous data were expressed as mean  $\pm$  standard deviation (SD). Continuous variables were compared using the Wilcoxon test. Categorical data were compared using Fisher's exact test. Risk factors with a univariate  $p$  value below 0.05 were included in a multiple regression analysis. A ROC curve analysis was performed to identify the cut-off for tumor size.

## **Results**

### *Study population and demographics*

From January 2005 to December 2007, 69 patients were enrolled for the study, including 38 males and 31 females, with a mean age of 67.4 ( $\pm 12.4$ ) years. Patients' demographic and pathological staging details are summarized in **table 1**. Four patients had two colon tumors. There were three pT4 tumors with microscopic invasion of the serosal layer not seen during surgery. Neo-adjuvant radiotherapy (45 Gy) and concomitant chemotherapy were given in 3 of the 16 rectal cancer patients. No complete pathological response was observed in the neo-adjuvant treatment group.



### *Results of SLN mapping*

The mean number of lymph nodes identified was 12.4 ( $\pm 7.0$ ). The mean number of sentinel nodes retrieved was 5.03 ( $\pm 4.2$ ). SLNM was successful in 71 (of 73) cases, with an identification rate of 97.3%. SLN mapping failed in a case of T2N0 right colon tumor (diameter 30 mm), and in a T3N0 sigmoid colon tumor. The SLN was the only site of metastasis in 8 cases, and 6 of these 8 SLNs only harbored micrometastases. Nine patients had false-negative SLNs, resulting in a false-negative rate of 31.0%. Mucinous features ( $p=0.01$ ), rectal cancer ( $p=0.02$ ), tumor size  $> 60$  mm ( $p=0.02$ ) and T3-4 tumors ( $p=0.05$ ) were all factors significantly associated with false-negative results on univariate analysis (**table 2**). False-negative patients also had a larger number of metastatic NSLNs. On multivariate analysis, rectal cancer (OR=6.2; 95% CI, 1.1 - 3.45) and mucinous features (OR=8.0; 95% CI, 1.4 - 45.3) were independently associated with false-negative findings (**table 3**).

### **Discussion**

The aim of this study was to establish the sentinel node detection rate and the prognostic factors influencing the false-negative rate in colorectal cancer. Ex-vivo SLN identification was done in the vast majority of patients by injecting patent blue dye into the submucosa of the tumor. The procedure was simple and inexpensive, and took only a few minutes for one of the surgeons to complete in the operating room, immediately after resecting the specimen.

The in-depth search on the few SLN identified and retrieved from each specimen enabled the pathologist to perform an extensive search for metastatic tumor cells in these nodes. By means of multiple sections and immunostaining, SLNs were identified as the sole site of metastases in 8 patients: most of these were micrometastases and would probably have gone undetected using a normal procedure, meaning that patients would have been denied adjuvant chemotherapy.

The second relevant finding of the study was the number of negative SLNs, despite metastatic disease being found in the NSLNs, - 9 in the group as a whole - leading to a false-negative rate of 31%. The main factors associated with false-negative findings in ex-vivo SLNM were rectal location, a mucinous histology, tumor stage (both T and N) and tumor size > 60mm.

A rectal tumor site is reportedly associated with higher false-negative SLN rates [16]. The mesocolon is much easier to inspect on both sides than the mesorectum, which may be fat and bulky. The rectal lymphatics are also more complex than their mesocolic counterpart: it may be that, in rectal cancer, a radioguided search (rather than a dye-driven search) might help to overcome these problems. There is no clear explanation for the diversity of behavior between non-mucinous and mucinous tumors: perhaps the presence of a mucinous lake alters normal lymphatic drainage.

Although patients with macroscopically-evident metastatic nodes were excluded from this study, the finding of numerous (> 3.9) metastatic NSLNs was associated with false-negative SLNs: this confirms previous reports that positive SLNs were less likely to be found in tumors with extra-nodal growth and gross lymph node invasion [17]. Extensive metastasis via lymphatic vessels may clog some lymphatic pathways, leading to the primary draining nodes being skipped and more distant nodes being invaded via secondary routes. Similarly, a bulky or locally-advanced tumor may interfere with sentinel node mapping, as shown by Patten and Saha [8,18]. False-negative SLNs in large tumors may also depend on the amount of dye injected, as shown by Viehl et al. [19]. In the present series, the amount of dye injected sufficed to color the submucosa around the tumor (up to 4 ml). It may be that using even larger amounts of dye could overcome this problem.

The search for SLNs in colon cancer has a completely different clinical goal from the case of breast cancer or melanoma (when SLNM is done to avoid any unnecessary extensive lymphadenectomy and to reduce the morbidity by yielding 1-2 prognostic nodes rather than the regional basin): in colon cancer, regional lymphadenectomy with en-bloc tumor resection remain the standard treatment, and the clinical problem is to yield the identification of any metastatic cells in retrieved nodes. The results of the present study confirm that pathological examination of ex-

vivo SLNs cannot be used as a short-cut for determining the pathological status of colon cancer since the method carries an unacceptable false-negative rate, ranging from 20% to 50% [20] (31% in this study). SLN identification enables the pathologist to focus on just a few nodes, and search these nodes extensively for metastatic cells, enabling the identification of metastatic cells in the category of patients who can benefit most from this more detailed search, i.e. those with small T1/T2 colon cancer. On the other hand, there is no point in searching for SLN in patients with advanced tumors because of the high false-negative rate and because finding positive SLN in such patients is unlikely to change the therapeutic strategy.

Two questions are still open and need to be investigated: 1. whether a more sophisticated radiolabeled SLN searching technology could make it easier to find SLN than using simple dye injection in rectal cancer; and 2. whether the random extensive examination of NSLNs might be as effective as examining SLNs. This issue was not addressed specifically in this study, but other authors recently showed that SLNs were significantly more likely to harbor micrometastases than NSLNs. [21,22].

In conclusion, sentinel lymph node mapping is feasible in colon cancer with a high identification rate: it enables metastases or micrometastases to be identified in a relevant number of patients, consequently inducing changes in treatment strategies. Since large tumors, extensive nodal metastases and a tumor location in the extraperitoneal rectum are associated with false-negative findings in SLNs, such patients could be excluded from the SLNM process, which appears to be more useful in the less-advanced stages of colon tumors. Further studies are needed to clarify the association between the mucinous variant and false negative findings in SLNs.

**Funding source**

The work was supported by a grant from the Regione Veneto, Ricerca sanitaria finalizzata, 2007.

**Acknowledgments:**

In memory of Prof. Carlo Tremolada (1944-2006)

ACCEPTED MANUSCRIPT

### References

1. Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Goodman PJ, Ungerleider JS, Emerson WA, Tormey DC, Glick JH, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. *N Engl J Med* 1990;322:352-8.
2. Impact B2 Investigators. Efficacy of adjuvant fluorouracil and folinic acid in B2 colon cancer. International Multicentre Pooled Analysis of B2 Colon Cancer Trials (IMPACT B2) Investigators. *J Clin Oncol* 1999 May;17:1356-63.
3. Le Voyer TE, Sigurdson ER, Hanlon AL, Mayer RJ, Macdonald JS, Catalano PJ, Haller DG. Colon cancer survival is associated with increasing number of lymph nodes analyzed: a secondary survey of intergroup trial INT-0089. *J Clin Oncol* 2003;21:2912-9.
4. Rodriguez-Bigas MA, Maamoun S, Weber TK, Penetrante RB, Blumenson LE, Petrelli NJ. Clinical significance of colorectal cancer: metastases in lymph nodes < 5 mm in size. *Ann Surg Oncol* 1996;3:124-30.
5. Cawthorn SJ, Gibbs NM, Marks CG. Clearance technique for the detection of lymph nodes in colorectal cancer. *Br J Surg* 1986;73:58-60.
6. Braat AE, Oosterhuis JW, de Vries JE, Tollenaar RA. Lymphatic staging in colorectal cancer: pathologic, molecular, and sentinel node techniques. *Dis Colon Rectum* 2005 Feb;48:371-83.
7. Joosten JJ, Strobbe LJ, Wauters CA, Pruszczyński M, Wobbles T, Ruers TJ. Intraoperative lymphatic mapping and the sentinel node concept in colorectal carcinoma. *Br J Surg* 1999;86:482-6.
8. Saha S, Wiese D, Badin J, Beutler T, Nora D, Ganatra BK, Desai D, Kaushal S, Nagaraju M, Arora M, Singh T. Technical details of sentinel lymph node mapping in colorectal cancer and its impact on staging. *Ann Surg Oncol* 2000;7:120-4.
9. Wood TF, Saha S, Morton DL, Tsioulis GJ, Rangel D, Hutchinson W Jr, Foshag LJ, Bilchik AJ. Validation of lymphatic mapping in colorectal cancer: in vivo, ex vivo, and laparoscopic techniques. *Ann Surg Oncol* 2001;8:150-7.
10. Veronesi U, Paganelli G, Galimberti V, Viale G, Zurrida S, Bedoni M, Costa A, de Cicco C, Geraghty JG, Luini A, Sacchini V, Veronesi P. Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph-nodes. *Lancet* 1997;349:1864-7.
11. Morton DL, Wen DR, Wong JH, Economou JS, Cagle LA, Storm FK, Foshag LJ, Cochran AJ. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg* 1992;127:392-9.
12. Saha S, Seghal R, Patel M, Doan K, Dan A, Bilchik A, Beutler T, Wiese D, Bassily N, Yee C. A multicenter trial of sentinel lymph node mapping in colorectal cancer: prognostic implications for nodal staging and recurrence. *Am J Surg* 2006;191:305-10.

13. Bertagnolli M, Miedema B, Redston M, Dowell J, Niedzwiecki D, Fleshman J, Bem J, Mayer R, Zinner M, Compton C. Sentinel node staging of resectable colon cancer: results of a multicenter study. *Ann Surg* 2004;240:624-8; discussion 628-30.
14. Greene FL. *AJCC Cancer Staging Handbook: TNM Classification of Malignant Tumors*. Springer 2003.
15. Hermanek P, Hutter RV, Sobin LH, Wittekind C. International Union Against Cancer. Classification of isolated tumor cells and micrometastasis. *Cancer* 1999;86:2668-73.
16. Braat AE, Oosterhuis JW, Moll FC, de Vries JE, Wiggers T. Sentinel node detection after preoperative short-course radiotherapy in rectal carcinoma is not reliable. *Br J Surg* 2005;92:1533-8.
17. Wong JH, Steineman S, Calderia C, Bowles J, Namiki T. Ex vivo sentinel node mapping in carcinoma of the colon and rectum. *Ann Surg* 2001;233:515-21.
18. Patten LC, Berger DH, Rodriguez-Bigas M, Mansfield P, Delpassand E, Cleary KR, Fagan SP, Curley SA, Hunt KK, Feig BW. A prospective evaluation of radiocolloid and immunohistochemical staining in colon carcinoma lymphatic mapping. *Cancer* 2004;100:2104-9.
19. Viehl CT, Hamel CT, Marti WR, Guller U, Eisner L, Stammberger U, Terracciano L, Spichtin HP, Harder F, Zuber M. Identification of sentinel lymph nodes in colon cancer depends on the amount of dye injected relative to tumor size. *World J Surg* 2003;27:1285-90.
20. de Haas RJ, Wicherts DA, Hobbelink MG, Borel Rinkes IH, Schipper ME, van der Zee JA, van Hillegersberg R. Sentinel lymph node mapping in colon cancer: current status. *Ann Surg Oncol* 2007;14:1070-80.
21. Stojadinovic A, Nissan A, Protic M, Adair CF, Prus D, Usaj S, Howard RS, Radovanovic D, Breberina M, Shriver CD, Grinbaum R, Nelson JM, Brown TA, Freund HR, Potter JF, Peretz T, Peoples GE. Prospective randomized study comparing sentinel lymph node evaluation with standard pathologic evaluation for the staging of colon carcinoma: results from the United States Military Cancer Institute Clinical Trials Group Study GI-01. *Ann Surg* 2007; 245:846-57.
22. Bembenek AE, Rosenberg R, Wagler E, Gretschel S, Sendler A, Siewert JR, Nährig J, Witzigmann H, Hauss J, Knorr C, Dimmler A, Gröne J, Buhr HJ, Haier J, Herbst H, Tepel J, Siphos B, Kleespies A, Koenigsrainer A, Stoecklein NH, Horstmann O, Grützmann R, Imdahl A, Svoboda D, Wittekind C, Schneider W, Wernecke KD, Schlag PM. Sentinel lymph node biopsy in colon cancer: a prospective multicenter trial. *Ann Surg* 2007; 245:858-63.

Patients	69		
Male/female ratio	1.2	(38/31)	
Mean age (yrs)	67.4	SD±12.4	
Cases	73	4 double tumors	
Colon/rectum cancer ratio	3.5	(57/16)	
	<b>Colon</b>	<b>Rectum</b>	<b>Total</b>
T			
T1	3	1	4
T2	13	5	18
T3	38	10	48
T4	3	0	3
N			
N0	43	9	52
N1	11	5	16
N2	3	2	5
Mean total lymph nodes	12.5 SD±7.7	11.7 SD±7.7	12.4 SD±7.0
Mean sentinel lymph nodes	5.3 SD±4.3	3.2 SD±2.1	5.0 SD±4.2

**Table 1.** Patients and pathological results

ACCEPTED MANUSCRIPT



Factors	False negatives (N=9)	All others (N=62)	<i>p</i> <i>value</i>
Rectum/Colon	5/4	11/51	0.02
Pre-operative CRT	1/5	2/11	NS
Polypoid/ulcerated	2/9	12/62	NS
Tumor size (mm)	52.9 (30-80)	37.7 (24-50)	NS <sup>^</sup>
Tumor size >60mm	6/3	3/59	0.02
Mucinous tumors	6/3	14/48	0.01
Grading G3/G2	0/9	6/56	NS
Lymphatic/Venous invasion	1/8	6/56	NS
T3-4/T2-1	9/0	41/21	0.05
N1/N2	6/3	7/2*	NS
Metastatic non-sentinel nodes	3.1 (1-9)	0.8 (0-4)*	0.01 <sup>^</sup>

**Table 2.** Factors relating to false-negative results on univariate analysis. ^Wilcoxon test\* Positive cases only.

ACCEPTED MANUSCRIPT

	OR	95% CI
Rectum/Colon	6.2	(1.1-3.45)
Mucinous tumors	8.0	(1.4-45.3)
Tumor size >60mm	6.4	(0.8-47.9)

ACCEPTED MANUSCRIPT

**Table 3.** Multiple logistic regression analysis for factors related to false negative results

ACCEPTED MANUSCRIPT