

**Specificity of an Individual Reference Limit (IRL)  
Compared with Conventional Cut-off during Intensive  
Post-operative Monitoring of Disease-free Breast  
Cancer Patients with serum CEA-TPA-CA15.3  
Tumor Marker Panel**

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## **Intensive post-operative breast cancer follow-up with serum tumor markers**

*“Early” treatment and standardised criteria for the use of serum tumour markers*

Current guidelines do not recommend routine use of laboratory or conventional instrumental examinations for post-operative surveillance of breast cancer patients.

Our and other authors' data (Jager W, Eur J Cancer Prev, 1993; Kovner F et al, Cancer Chemother Pharmacol, 1994; Nicolini A et al, Br J Cancer, 1997) on significantly prolonged relapse-free survival (RFS) and/or overall survival (OS) and some theoretical reasons arise the principal question of the importance of an “early” detection and treatment of metastatic disease by serum tumor markers.

However, so far no large prospective randomised trial has been conducted to definitely answer this question.

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Meta-analysis of most recent reports from scientific literature on the performance of one or more markers in the “early” detection of breast cancer relapse.

Specificity and sensitivity of serum tumor markers (TM) in the meta-analysis according to the number of the evaluated tumor markers.

TM (n)	Specificity (%)			Sensitivity (%)		
	Range	Median	Difference % between medians	Range	Median	Difference % between medians
1	90-98	96		33-69	54	
2	82.5-95	88	-8 (1 vs 2)	61-88	78.5	+24 (1 vs 2)
3	79-91	84	-12 (1 vs 3)	85-95	90	+36 (1 vs 3)

Besides, dynamic evaluation showed better performance than single positive/negative cut-off value.

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Different common criteria used in the meta-analysis for evaluation of serum tumor marker sampling.

Technique	Cut-off		Critical change (CC)	
	Source	Value	Value	Method
EIA	Commercial kit	Commercial kit	> cut-off (single positive/negative cut-off value)	Mathematical
IRMA	Commercial kit	Commercial kit increased	2 high sequential values in 2 months or 2 sequential progressively increasing values	Observational
RIA	Controls	95° percentile	25% > cut-off	Mathematical
ELISA	Patients	Individual reference limit	Constant elevation or progressive increase	Observational
			Elaborated criteria	Mathematical
			Rising or fluctuating levels above cut-off	Observational

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The absence of standardised criteria is likely the main obstacle to carry out prospective multicenter randomised clinical trials using serum tumour markers as “early” signal of distant metastases.

A mathematical definition of reference change value (RCV) was proposed.

$RCV = z \times 2^{1/2} \times \sigma$  within-subject

(Harris and Yasaka, 1983)

When  $z = 1.96$ :  $RCV = 2.77 \times \sigma$  within-subject

But in 2008, as to RCV, Petersen et al. stated that “a tool for better understanding and interpretation... is needed” and proposed “likelihood ratio (LR+), pre-tests and post-tests odds as supplement to RCV (Clin Chem Lab Med 2008, 157-164).

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Indeed, so far, any mathematically or observationally defined “critical change” (CC) neither spread within scientific community nor extended to current clinical practice.

This likely occurred as mathematically defined CCs are not easily applicable and observationally defined CCs are not easily reproducible.

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*Early” treatment and standardised criteria for the use of serum tumour markers*

In the past, as cut-off value we used that of the conventional kit and constant elevation (CE) and progressive increase (PI) as CC.

PI: when the tumor marker value is 30% or more higher in the sample taken 2-3 weeks following the initial elevated value.

CE: otherwise, 2 consecutive high values are regarded to be a CE.

Patients with CC (CE or PI) unexplained by any known concomitant benign pathology were suspected for a relapse.

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In 2008 we proposed a CC based on an individual reference limit (IRL) as cut-off value. Recently, this cut-off value was calculated as follows and it was planned to be adjusted every 2 years.

Five consecutive serum values from blood samples regularly withdrawn within 6 months at the beginning of the follow-up.

$$\text{IRL (cut-off value)} = \text{Mean} + 2 \text{SD}^*$$

\*this was the IRL when SD was  $\geq 20\%$  of Mean; otherwise, SD was taken as 20% of Mean.

The CC has been defined considering both mathematical criteria (within-subject biological and statistical variability) and observational findings (rising levels at the relapse).

		Cut-off		Critical change (CC)	
Technique	Source	Value	Duration	Value	Method
Any	Commercial kit	IRL	2 years	3 consecutive values $>$ IRL in blood samples withdrawn at 1-2 weeks interval time	Mathematical and observational

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Patients with constant elevation (CE) or progressive increase (PI) during post-operative intensive follow-up with CEA, TPA and CA15.3 tumor markers.

Reasons	CEA (1)		TPA (1)		CA15.3 (1)		CEA-TPA- CA15.3 (1, 2)
	CE %	PI %	CE %	PI %	CE %	PI %	Falsely positive pts %
CBP	1.7	0	17.3	8	1.7	0.6	n.a.
Unknown	0.6	0	1.7	0	2.3	0	n.a.
Total	2.3	0	19	8	4	0.6	4

(1) BJC 1991; (2) BMC Cancer 2006

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Serum values of CEA, TPA, CA15.3 and CEA-TPA-CA15.3 association higher than IRL during intensive post-operative monitoring of 124 disease-free breast cancer patients (follow-up 5.5 months, mean).

Tumor marker	D	Elevated D		Patients with elevated D		Falsely positive pts	
	n	n	%	n	%	n	%
CEA	266	2	0.7	1	0.8	0	0
TPA	266	52	19.5	35	28.2	1	0.8
CA15.3	266	4	1.5	3	2.4	0	0
CEA-TTPA-CA15.3 panel	266	57	21.4	38	30.6	1	0.8

D = determination

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0.8% falsely positive patients with CEA-TPA-CA15.3 panel during an intensive post-operative monitoring of 5.5 months (mean) roughly corresponds to 4% of falsely positive patients we have previously reported during an intensive post-operative monitoring of 32 months (mean). Nevertheless, with this new method using IRL as cut-off and 3 consecutive values higher than IRL as CC, the same specificity than with our old method has been obtained but no subjective interpretation on concomitant benign pathology was necessary.

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## **Conclusions**

In disease-free breast cancer patients, the new method we propose showed high specificity of serum CEA-TPA-CA15.3 panel. Moreover, unlike our old method and that from other Authors, it permits to standardize the use of serum tumor markers and likely to carry out in these patients an intensive monitoring with favourable cost-effectiveness ratio.

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