

1995 1998
keratin-19
RT-PCR 59
acid guanidine
phenol chloroform total RNA
keratin-19 RT primer
PCR (Table 1). RT-PCR
DNA 2% agarose gell
Total RNA control (-actin)

1) RNA

10 ml lymphoprep
(Nycomed) 2,700 rpm 20
-70°C . Trizol (Gibco
BRL) 가 total RNA
260 280 nm

2) Oligonucleotide primer

가 oligonucleotide primer human keratin 19 DNA
DNA external primer
set K 19ES (external sense) 5'-aggtggattccgctccgggca-3',
K 19EA (external antisense) 5'-atcttctgtccctcgagc-3' RT-
PCR 461
inner primer set K 19IS (internal sense) 5'-agtgtgtctccaagg-
cagc-3', K 19IA (internal antisense) 5'-gacatgccaagccaat-
atgagg-3' nested PCR 221
PCR (Table 1).

3) RT-PCR nested PCR

PCR (50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1
% Triton X-100, 200 uM dNTP, 2.5 mM MgCl2, 0.1 mM DTT,

Table 1. External and internal primer for keratin-19

External	5'-AGGTGGATTCCGCTCCGGGCA-3'' (739 759)
	5'-ATCTTCCTGTCCCTCGAGCA-3'' (1180 1199)
Internal	5'-GACATGCGAAGCCAATAIGAGG-3' (783 804)
	5'-AGTGTGTICTTCCAAGGCAGC-3'' (984 1003)
-actin	5'-CGCTGTGTTGGCGTACAGGT-3''
	5'-TCATCACCAATTGGCAATGAG-3''

*Underlying areas are different base from pseudogene.

60 U M-MLV reverse transcriptase, 0.5 U taq polymerase, 12
U RNase inhibitor, 10 mM sense primer, 10 mM antisense
primer) total RNA 1 µg DEPC DW
30 µl . DNA thermal cyclcer (Perkin
Elmer Cetus) 42°C 1 cDNA
denaturation (94°C, 1), annealing (57°C, 1.5),
extension (72°C, 2) PCR cycle 35
extension 10 RT-PCR .
2 µl nested PCR
phenol chloroform total RNA
keratin-19 RT primer
PCR (Table 1). RT-PCR
DNA 2% agarose gel
Total RNA control (-actin)
ethidium bromide UV illuminator
. mRNA
-actin RNA (Fig. 1).
MCF7 10 ml 10⁷
10⁵ 1
nested RT-PCR

4)

3
, 6
Mann-Whitney
Kaplan-
log-rank
Meier
SPSS 10.0 . P <
0.05

59 가
10⁷ 가 10 가
가 10⁶
가 (Fig. 2).

1) RT-PCR

DCIS 4 1 T1

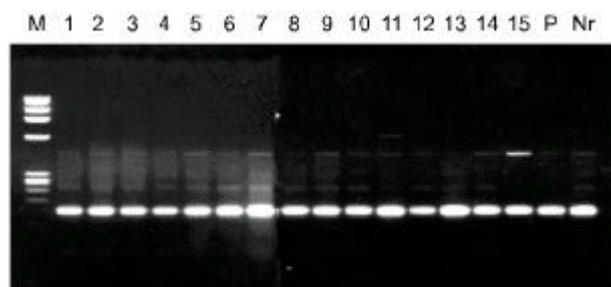


Fig. 1. Nested RT-PCR for beta actin. -actin shows positive result in all smpls.

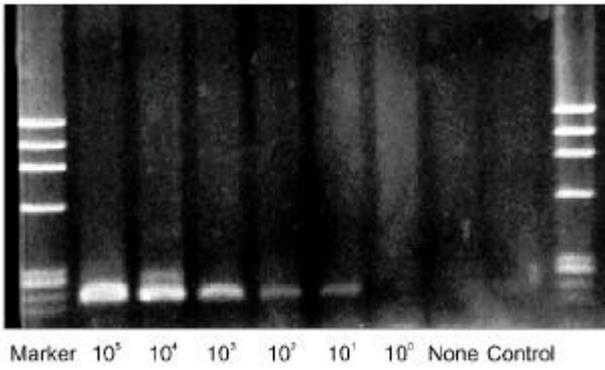


Fig. 2. Sensitivity test of RT-PCR. Nested RT-PCR for keratin-19 shows positive result in 10 cells in 10 million peripheral nucleated blood cells.

Table 2. Tumor staging and bone marrow micrometastasis

	BM mets (-)	BM mets (+)	Total	P-value
Tumor size				
DCIS	3	1	4	
T1	17	13	30	
T2	9	11	20	
T3	0	4	4	
T4	1	0	1	0.091
Node staging				
N0	21	20	41	
N1	5	6	11	
N2	4	3	7	1.00
Staging				
DCIS	3	1	4	
Stage I	15	11	26	
Stage II	9	13	22	
Stage III	3	4	7	0.148
Total	30	29	54	

*BM = bone marrow; mets = metastasis.

30	13	가	T2	20	11
			T3	4	
			T4	1	
			. N staging		
			N0	41	20 가
11	6		N2	7	3
			DCIS	4	1
			, 1	26	11 , 2
			13 , 3	7	4
2).					(Table

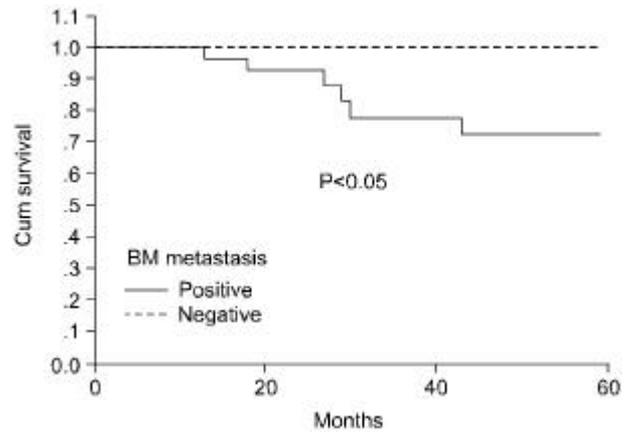


Fig. 3. Bone marrow micrometastasis and disease Free Survival. Disease free survival curves according to bone marrow micrometastasis were statistically significant difference between groups.

Table 3. Prognostic factors and tumor recurrence

	Recur (-)	Recur (+)	Total	P-value
Tumor size				
DCIS	4	0	4	
T1	29	1	30	
T2	16	4	20	
T3	2	2	4	
T4	1	0	1	0.004
Node staging				
N0	38	3	41	
N1	9	2	11	
N2	5	2	7	0.088
Stage				
DCIS	4	0	4	
Stage I	26	0	26	
Stage II	18	4	22	
Stage III	4	3	7	0.002
Bone marrow micrometastasis				
BM mets (-)	30	0	30	
BM mets (+)	22	7	29	0.004
Total	52	7	59	

2)		RT-PCR	
59	7	DCIS	4
		T1	30
		가	T2
20	4	T3	4
		2	
		T4	1
		N0	41
		3	, N1

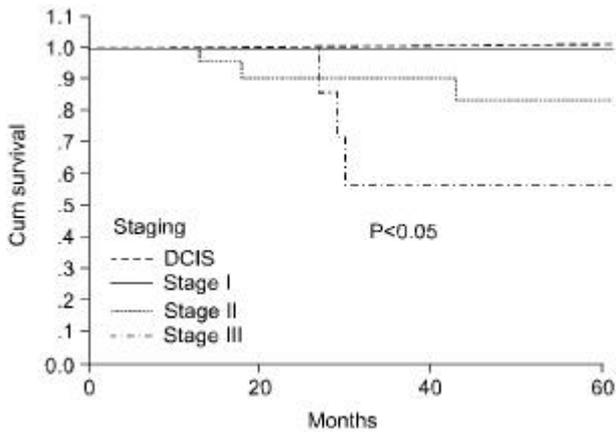


Fig. 4. Tumor staging and disease Free Survival. Disease free survival curves according to stage were statistically significant difference between groups.

Table 4. Prognostic value of disease free survival curves according each factors by log rank test

Prognostic factors	P-value
Bone marrow micrometastasis	0.004
Lymph node metastasis	0.08
Staging	0.01
Lymphovascular invasion	0.017
Histologic grade	0.24
Estrogen receptor	0.73

11 2 , N2 7 2
 DCIS 4 1 26
 , 2 22 4 , 3 7 3 가
 Keratin 19 RT-PCR
 RT-PCR 30 RT-PCR 29
 7 (Table 3).
 log rank test
 60
 (P=0.004) (Fig. 3).
 (P=0.01) (Fig. 4)
 (Table 4).

(4-7)
 , RT-PCR
 가
 가
 가
 (8)
 Keratin 19
 8, 18, 19 keratin 8, 18
 keratin 19
 가
 Deamaley (9)
 가
 가 24 10
 가
 23%
 9.5
 85%가 가 31%
 가 (11, 12) RT-PCR
 mRNA DNA
 Shoenfeld
 Kruger 가
 24 (13, 14) Vannuchi (15) 14
 Braun (16) 150

RT-PCR
 Keratin 19 pseudogene 가 가
 pseudogene Keratin 19 가
 가 (19-23)
 Primer가 external primer sense primer 2
 nucleotide (749, 754)가 antisense 3
 nucleotide pseudogene internal keratin 19
 primer sense primer 4 (783,793,794,800
), antisense primer 2 nucleotide
 pseudogen 가
 pseudogene primer
 pseudogene
 (Table 1). Keratin 19 keratin 8,18

nested RT-PCR RT-PCR
 primer primer
 PCR
 Nested RT-PCR
 42% 49%
 RT-PCR
 (24,25)
 RT-PCR
 30% 51%가
 sensitivity test
 DCIS 4 1
 RT-PCR
 RT-PCR
 가
 2 3 가
 가
 (26)
 가
 가 가 가
 가 mammo-
 globlin

mammoglobin MCF
 가
 7
 (17,18) (27,28)
 가 가 가
 가
 가
 nested RT-PCR
 keratin 19
 DCIS
 가 가
 RT-PCR
 가
 가

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