Survival Time among Young and Old Breast Cancer Patients in Relation to Circulating Blood-Based Biomarkers, Acute Radiation Skin Reactions, and Tumour Recurrence

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Keywords

Survival time · Blood-based biomarkers · Acute radiation skin reactions · Tumour recurrence

Abstract

Introduction: It has been suggested that age could influence the treatment-induced side effects and survival time of cancer patients. The influence of age on blood-based biomarkers, acute radiation skin reactions (ARSRs), and survival time of breast cancer patients was analysed. **Materials and Methods:** Two hundred ninety-three individuals, 119 breast cancer patients, and 174 healthy blood donors were included. **Results:** Before radiotherapy (RT), decreased levels of lymphocytes, interleukin 2, platelet-derived growth factors, and tumour necrosis factor but increased levels of monocyte-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, C-reactive protein, and macrophage inflammatory protein 1b (MIP1b) were detected in the patient group. All of the patients developed ARSRs and intensity of ARSRs was inversely related to the MIP1b level before RT. Fifteen out of 119 (13%) patients deceased during follow-up time. No influence of age (≤ 50 compared to > 50 years) on survival time was detected ($p = 0.442$). Tumour recurrence, found in 11 out of 119

(9%) patients, had impact on survival time of these patients ($p < 0.001$). **Conclusions:** The level of circulating MIP1b before RT was associated with intensity of ARSRs. Tumour recurrence, but not age, was associated with poor survival time. Analysis of circulating MIP1b was low cost, rapid, and could be done in routine laboratory facility. Since RT almost always induces ARSRs, the possibility of using MIP1b as a prognostic biomarker for ARSRs is of interests for further investigation.

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Introduction

Aging could be associated with a decline of host immunological status that might influence the risk of various diseases and treatment outcome of patients [1–4]. With improved sanitary conditions, health-care systems, nutrition and lifestyle, life expectancy of elderly patients has increased worldwide [5].

At low cost, automatic and rapid detection of host immune status could be done by analysing circulating blood-based biomarkers. These biomarkers could be lymphocytes, monocytes, neutrophils, plasma C-reactive protein

(CRP), cytokines, and chemokines [6, 7]. Independent of tumour-node-metastasis staging and age, plasma CRP levels, and distribution of white blood cell subpopulations before treatment were associated with clinical outcome of cancer patient [8–11].

Breast cancer is the most diagnosed cancer among women worldwide and is the leading cause of death from cancer among females [12, 13]. Variation of blood-based biomarkers in breast cancer patients after surgery was reported [14]. Adjuvant radiotherapy (RT) is an essential part of breast cancer treatment after surgery. The anti-neoplastic properties of RT are primarily related to DNA damage and cell death. Direct and indirect effects of RT could lead to alterations in host immune response and acute radiation skin reaction (ARSR) during the treatment [15, 16]. The ARSR develop in the normal tissue and could be manifested as erythema and dry or moist desquamation. These reactions are often associated with discomfort, itching, pain, and disturbed sleeping pattern [17, 18]. In serious cases, ARSR could be therapy limiting in the patients.

The radiation therapy oncology group (RTOG) scoring system for acute dermatitis is often used as indicator of ARSR intensity [19]. Prognostic biomarkers for prediction of individual ARSR could be of value for the individual patient treatment selection in the clinical practice.

The present study focusses on age, blood-based biomarkers, and 5 to 9 years clinical outcome of breast cancer patients after completed RT. Our clinical outcome was ARSR, tumour recurrence, and survival of the patients during the follow-up time.

Materials and Methods

Patients and Controls

The participants in this prospective study were women from a community-based population of European descent in the Jönköping region, Sweden. Female breast cancer patients older than 19 years, scheduled for standard adjuvant RT after breast cancer surgery at Department of Oncology, Ryhov hospital, Sweden, were invited to participate.

Female healthy blood donors in the Jönköping region older than 19 years with no history of previous cancers or any use of immunomodulation agents were invited to participate as controls. If the patients before RT or controls accepted the invitation, 30 mL venous blood was drawn, using EDTA tubes. A total of 119 patients and 174 controls were included during the period 2011 through 2015. Informed consent was obtained from all participants.

Blood-Based Circulating Biomarker

The levels of circulating lymphocytes, monocytes, and neutrophils were analysed using whole blood samples with a Sysmex

Table 1. Characteristics of 119 female breast cancer patients

	<i>n</i>
Tumour stage	
<T1	73
>T2	34
Tis	12
Lymph node status	
<N0	82
>N1	37
Histology	
DCIS	12
Ductal	80
Lobular	16
Others	11
Receptor status	
Estrogen+	100
Progesterone+	81
Her2+	10
Age, years	
<50	15
>50	104

XE5000 instrument (Sysmex Corporation, Kobe Japan). Based on previous investigations, plasma CRP, interleukin (IL) 2, IL12, platelet derived growth factors (PDGF), macrophage inflammatory protein 1b (MIP1b), and tumour necrosis factor α were analysed [9, 10, 20]. Customised fluorochrome kits for the multiplex fluorochrome technique (Luminex xMAP™ Technology, Austin, TX and Bio-Rad Laboratories, Hercules, CA) was used for detection of plasma protein, cytokines, and chemokines. Circulating high-sensitive CRP levels in the plasma were analysed using Siemens Advia 1800 (Siemens Healthcare, Erlangen, Germany) with reagents and protocols from the company.

Adjuvant RT, ARSR, Tumour Recurrence, and Survival Time

Adjuvant RT was given at the Department of Oncology, Ryhov Hospital, Sweden, using a Varian True Beam instrument (Varian, Palo Alto, CA). The patients received standard treatment with a total of 50 Gy to the operated breast in 25 fractions, one fraction per day administered 5 times per week for a total of 5 weeks.

The patient who developed early erythema of the skin during RT received potent steroid cream betamethasone on the radiated breast. This additional steroid cream treatment will be continued up to 3 weeks after completed the RT.

Intensity of ARSR during RT was recorded based on the patient experiences by a special nurse [16]. The ARSR was grouped as mild (RTOG score 0 and 1) or high (RTOG score ≥ 2) according to their ARSR intensity. Tumour recurrence and survival of the patients during a follow-up time of 5 to 9 years after completing RT were collected from the patient medical record.

Statistical Analysis

Descriptive statistics, means, numbers, and percentages were presented where suitable. Student's *t* test was used to compare the level of the blood-based biomarkers among and between the pa-

Table 2. Circulating blood-based biomarkers in 119 breast cancer patients and 173 controls: comparison between the control and patient group

	Controls (n = 173)	Patients (n = 119)	p value*	Age ≤50 years			Age >50 years		
				controls (n = 33)	patients (n = 15)	p value*	controls (n = 140)	patients (n = 104)	p value*
Lymphocytes, 10 ⁹ /L	1.96	1.68	<0.0001	1.91	1.49	0.0096	1.98	1.7	0.0003
Monocytes	0.53	0.56	0.197	0.58	0.58	0.95	0.52	0.56	0.122
Neutrophils	3.59	3.93	0.055	3.95	4.00	0.91	3.50	3.92	0.0284
MLR	0.28	0.36	0.0001	0.33	0.41	0.077	0.28	0.36	0.0002
NLR	1.96	2.55	<0.0001	2.27	2.87	0.14	1.88	2.51	<0.0001
CRP, mg/L	2.22	4.26	<0.0001	2.68	3.07	0.71	2.11	4.43	<0.0001
IL2, pg/mL	21.64	14.96	0.013	20.15	9.19	0.004	21.97	15.7	0.041
PDGF, pg/mL	805.1	604.6	0.007	696.7	430.70	0.034	829.10	626.7	0.015
MIP1b, pg/mL	44.34	55.97	0.0005	39.30	52.71	0.041	45.45	56.39	0.0038
TNFa, pg/mL	96.17	66.40	<0.0001	94.25	53.67	0.11	96.57	68.03	0.0005
IL12, pg/mL	33.86	29.63	0.42	34.45	17.34	0.019	33.73	31.19	0.667

PDGF, platelet-derived growth factors; CRP, C-reactive protein; MIP1b, macrophage inflammatory protein 1b; TNFa, tumour necrosis factor a; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; IL, interleukin. * p value after Benjamini and Hochberg adjustment.

tients and controls. Stratified by age, patients, and controls were divided into ≤50 years (young) and >50 years (old) groups. The association of ARSR intensity according to RTOG and the level of blood-based biomarkers in the young and old groups was analysed. To evaluate the survival time after completing RT and the effect of tumour recurrence on survival, Kaplan-Meier estimations were used.

SAS® stat version 13.1 software and Proc Life test by SAS 9.4 software (SAS Institute Inc., Cary, NC) were used for the statistical survival analysis. To reduce the number of false-positive values, Benjamini-Hochberg correction for multiple testing was done [21]. The threshold level was set at a $p \leq 0.05$ for a statistically significant value after correction for multiple testing.

Results

Patients and Healthy Controls (Table 1)

A total of 293 individuals were included in this study. They were 119 female breast cancer patients, with a median age of 64 (range 31–86) years. The patients were prospectively included. There was heterogeneity regarding tumour stage, lymph node status, tumour location, and receptor expression (Table 1).

The controls comprised 174 female healthy blood donors, with median age of 55 (range 21–89) years. The median age of the patient group was 9 years higher than of the control group, due to the age restrictions for being blood donors.

Circulating Blood-Based Biomarkers in the Patients before RT and Controls (Tables 2 and 3)

A lower level of lymphocytes, IL2, PDGF, and tumour necrosis factor but a higher monocyte-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, CRP, and MIP1b were found among the patients compared to the controls. Despite variation in age, lower levels of lymphocytes, IL2, and PDGF but a higher level of MIP1b were found among the old and young patients compared to their age-matched controls.

Old age patients (>50 years) had higher levels of neutrophils, monocyte-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, and CRP than the old age controls. Only the level of IL12 was lower among young patients (≤50 years) than the young age controls.

No statistically significant difference in levels of these biomarkers was observed between young and old controls. Only the level of IL12 was higher among old patients than young patients.

ARSR and Circulating Blood-Based Biomarkers of the Patients before RT (Table 4)

During RT, the patients developed ARSR (Table 4). Low ARSR (RTOG 0 and 1) were detected in 57 patients (48%) and high ARSR (RTOG ≥2) were detected in 62 patients (52%). Out of 62 high ARSR patients, 59 patients received steroid cream at early onset of erythema. Despite steroid treatment, 51 patients (86%) developed ARSR

Table 3. Circulating blood-based biomarkers in 119 breast cancer patients and 173 controls: comparison between the younger and older age groups within controls or patients

	Controls			Patients		
	age ≤50 years (n = 33)	age >50 years (n = 140)	p value*	age ≤50 years (n = 15)	age >50 years (n = 104)	p value*
Lymphocytes	1.91	1.98	0.534	1.49	1.71	0.129
Monocytes	0.57	0.52	0.106	0.58	0.56	0.769
Neutrophils	3.94	3.50	0.116	4.00	3.92	0.859
MLR	0.33	0.28	0.0474	0.41	0.36	0.369
NLR	2.26	1.89	0.115	2.86	2.51	0.303
CRP, mg/L	2.68	2.11	0.322	3.07	4.43	0.291
IL2, pg/mL	20.15	21.97	0.578	9.19	15.70	0.055
PDGF, pg/mL	696.70	829.10	0.341	430.70	626.70	0.373
MIP1b, pg/mL	39.31	45.46	0.174	52.71	56.39	0.605
TNFα, pg/mL	94.26	96.58	0.861	53.67	68.03	0.182
IL12, pg/mL	34.46	33.74	0.924	17.34	31.19	0.0334

PDGF, platelet-derived growth factors; CRP, C-reactive protein; MIP1b, macrophage inflammatory protein 1b; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; IL, interleukin. *p value after Benjamini and Hochberg adjustment.

Table 4. Circulating blood-based biomarkers and degree of ARSR (RTOG score) in breast cancer patients

	RTOG 0+1 (n = 57)	RTOG ≥2 (n = 62)	p value*	Age ≤50 years			Age >50 years		
				RTOG 0+1 (n = 7)	RTOG ≥2 (n = 8)	p value*	RTOG 0+1 (n = 50)	RTOG ≥2 (n = 54)	p value*
Lymphocytes, 10 ⁹ /L	1.68	1.69	0.928	1.47	1.51	0.874	1.71	1.71	0.99
Monocytes	0.55	0.57	0.494	0.56	0.60	0.666	0.55	0.57	0.558
Neutrophils	3.87	3.99	0.654	4.29	3.66	0.358	3.80	4.04	0.436
MLR	0.34	0.39	0.215	0.39	0.43	0.650	0.34	0.38	0.23
NLR	2.45	2.65	0.376	3.03	2.67	0.595	2.35	2.65	0.222
CRP, mg/L	4.47	4.05	0.619	3.39	2.64	0.711	4.66	4.22	0.63
IL2, pg/mL	15.65	14.31	0.73	6.80	12	0.31	16.92	14.58	0.587
PDGF, pg/mL	653.8	557.8	0.341	429.5	432.10	0.987	685.90	572.10	0.308
MIP1b, pg/mL	62.39	49.89	0.0055	57.84	46.73	0.349	63.04	50.25	0.0092
TNFα, pg/mL	72	61.10	0.273	45.42	63.30	0.337	75.79	60.86	0.172
IL12, pg/mL	28.22	30.96	0.752	13.60	21.70	0.346	30.31	32.01	0.860

PDGF, platelet-derived growth factors; ARSR, acute radiation skin reaction; CRP, C-reactive protein; RTOG, radiation therapy oncology group; MIP1b, macrophage inflammatory protein 1b; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; IL, interleukin. *p value after Benjamini and Hochberg adjustment.

score RTOG 2 and 8 patients (14%) developed an ARSR score >2.

Stratified by age, high ARSR was found among 8 out of 15 (53%) young patients and 54 out of 104 (52%) old patients. These differences were not statistically significant.

Out of the studied biomarkers, only the MIP1b level before RT was different between the patients with low and high intensity of ARSR, especially among old patients. The levels of MIP1b were inversely related to the intensity of ARSR in these old patients (Student's *t* test, *p* < 0.006).

Fig. 1. Survival time and tumour recurrence. **a** Survival time in the tumour recurrence and non-recurrence group. **b** Survival time after RT of the young and old patient group. RT, radiotherapy.

Tumour Recurrence and Survival Time (Fig. 1a, b)

During the follow-up time of 5 to 9 years after RT, tumour recurrence was detected among 11 out of 119 (9%) patients. The recurrence occurred in one out of 15 (7%) young patients and 10 out of 104 (10%) old patients. Numbers of deceased patients were 15 out of 119 (13%) during the follow-up time.

In the tumour recurrence group, 9 out of 11 (82%) patients died, whereas 6 out of 108 (6%) died in the non-recurrence group (log-rank test, $p < 0.001$, Fig. 1a). The median survival time after recurrence was 5 years.

Stratified by age, one out of 15 (7%) young patients and 14 out of 104 (14%) old patients died during the follow-up time (Fig. 1b). There was no difference in survival time of young patients compared to old patients (log-rank test, $p = 0.442$).

Discussion

Worldwide increasing life expectancy of healthy population was reported [3, 5]. At the studied age group in our investigation, aging per se has a marginal influence on the studied blood-based circulating biomarkers in healthy individuals. Thus, chronological age is not a good indicator of an individual biological age status.

Our investigation included breast cancer patients with small-size tumours at relatively early stage of disease. Despite early detection, pathological conditions influence blood-based biomarkers in the patients. Microphage in-

flammatory protein 1 beta (MIP 1b), also known as CCL4, is a chemotactic cytokine that mediate host inflammatory responses, leukocyte migration and activities of other immune response cells [22–24]. MIP1b is highly expressed in breast tumour cells and various cells in the tumour microenvironment but are minimally expressed in normal breast epithelial cells [25]. MIP1b could promote tumour development and progression by recruiting regulatory T cells [26].

In spite of using a potent steroid cream at early onset of erythema, all of these patients developed ARSR score ≥ 2 . Thus, additional use of steroid cream for reducing ARSR needs further investigation.

Here, we report that the circulating level of MIP1b before RT had a reversed association with intensity of ARSR among the old patient group. The lack of association found in young patients might be due to a low number of patients in this group. Blood-based MIP1b analysis is low cost, rapid, and could be done in a routine laboratory facility. The possibility of using blood-based MIP1b as a predictor of ARSR intensity in the clinic requires further investigation.

The strength of our study was that the patients and controls were included from a population in one geographic region. All patients received standard RT were followed up by one health-care person in one clinic. The study limitation was a relatively small number of included young patients. Our control group has lower median age than the patient group. No statistically significant differences in the level of the investigated biomarkers were observed between

young and old controls. This suggested that the control group was suitable to use in our investigation.

With the Swedish health care and the effective screening system, a minimum of 5 up to 9 years follow up, might not be adequate for detection the impact of aging on survival among the breast cancer patients [27]. In addition, the patient's lifestyles, such as smoking, drinking, obesity, or physical activity and heredity factor, need to be considered.

Conclusion

Our results indicated that circulating levels of MIP1b before RT were reversely associated with the intensity of ARSR among older patients. Chronological age had no influence on the investigated blood-based biomarkers among healthy controls.

Tumour recurrence was associated with poorer patient outcome, independent of ARSR and age. The relatively low number of deceased patients in our study might be due to early detection of tumour, efficiency of surgery and RT, or the combination of these parameters.

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Statement of Ethics

The study was conducted in accordance with the Declaration of Helsinki for studies of humans. The informed written consent was obtained from all accepted participants. The Ethical Board at Linköping University approved this investigation (Dnr 2010/331-31).

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest to disclose regarding this study.

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Author Contributions

N.L.: conception, design of the investigation, interpretation of results, and draft of the manuscript. D.O.: acquisition the patients, observed the acute radiation skin reaction, and all clinical information. M.N.: statistical analysis of laboratory and clinical data. B.A.: establishing the methods and conduct the laboratory analysis. S.L.: conception and interpretation of laboratory results. F.L.: conception, design of the investigation, follow-up the patient clinical outcome, and interpretation of clinical results. All authors contributed significantly to write the final version.

Data Availability Statement

Raw data were generated at Department of Laboratory Medicine and Department of Oncology, Ryhov hospital, Sweden. Derived data supporting the findings of this study are available from the corresponding author on request.

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