Chapter 8

Comparison of distribution and prognostic effect of adaptive tumor immune subtypes between young and elderly breast cancer patients

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Submitted

ABSTRACT

Purpose: As demonstrated recently, tumor immune subtypes, representative for various tumor immune control and host immune escape phases, are a strong prognostic factor for breast cancer outcome. With ageing, immunosenescence occurs, which might impair tumor immune surveillance.

Experimental Design: All non-metastasized breast cancer patients primarily treated with surgery in the Leiden University Medical Center between 1985 and 1996 were included (n=714). Tumor immune subtypes were previously categorized in groups of increasing immune susceptibility using quantifications of immunohistochemically stained tumor infiltration of CD8+ cells, natural killer cells, T regulatory cells, and tumor expression of HLA class I and HLA EG. Associations between immune markers and age at diagnosis (<65 versus >=65 years) and outcome analyses according to age were performed.

Results: A statistically significant association was found between less HLA-EG upregulation and patients aged >=65 years ($p=0.015$). In addition, though not significant, less low immune susceptible tumors were seen in these older patients. In patients aged <65 tumor, higher immune susceptibility resulted in statistically significant favorable patient outcome independently of known clinicopathological parameters (RFP $p<0.001$, RS $p<0.001$). In patients aged ≥65, immune subtypes showed no statistically significant association with outcome.

Conclusions: Less immune susceptible tumors were found in elderly breast cancer patients, supporting the idea of immunosenescence potential role in cancer progression. In addition, contrary to the results found in patients aged <65 years, no statistically significant association was found between tumor immune subtypes and patient outcome in patients aged >65 years. A better understanding of processes of immunosenescence and tumor progression and future possibilities in immune manipulations and vaccinations might lead to more tailored treatment of elderly breast cancer patients.

INTRODUCTION

Breast cancer is the leading contributor to cancer incidence and cancer mortality in women worldwide, with 1.383.500 new cases in 2008 (1). Nearly one third of these breast cancer patients are 65 years or older (2). As breast cancer incidence increases with increasing age, changing demographics and continuously increasing life expectancy will further enlarge the number of elderly women confronted with breast cancer. A recent report observed that regardless of a higher risk of mortality from other causes and independent of known tumor and patient characteristics, mortality from breast cancer increased with age (3) Cancer immune surveillance and immunosenescence at increasing age, may contribute to an explanation of this finding.

There has been strong evidence that the host's immune system is able to control tumor progression (4). On the other hand, due to their intrinsic genetic unstable nature, tumor cells may acquire properties to escape from such immune recognition (5). Various interactions underlie this balance between tumor immune control and escape (Figure 1A). Cytotoxic T-lymphocytes (CTL) are capable of recognising tumor associated antigens presented by classical human leukocyte antigen (HLA) class I (HLA-A, HLA-B, HLA-C) on the tumor cell surface. In order to avoid immune recognition from CTL, cancer cells may lose expression of classical HLA class I (6). However, this makes them prone to natural killer (NK) cell recognition (7). Non-classical HLA class I molecules (HLA-E, HLA-G) play a crucial role in immune surveillance by NK-cells. Expression of these molecules on the cell surface causes an inhibitory effect on NK-cell attack (7-9). Another known tumor escape mechanism is the attraction and induction of immune suppressive regulatory T cells (Treg) in the tumor microenvironment (10). There is evidence for a variety of these immune reactions in breast cancer, where it has been shown that breast cancer is highly immunogenic (11, 12), but also capable of evading immune recognition. (13, 13-21) This emphasizes the importance of taking into account the various interactions which exist between the tumor and the immune system. We recently defined tumor immune subtypes, based on the above mentioned immunological interactions, which were shown to be a highly discriminative prognostic biomarker with solid underlying biological rationale (22).

With increasing age, there appears to be a progressive accumulation of cellular and molecular alterations leading to tissue dysfunction. This equally applies to the immune system, where the age-related decline of functional innate and adaptive immunity leads to a reduced ability to respond to infection, vaccinations or cancer (23, 24). Though the exact mechanisms of immunosenescence are not fully understood, various phenomena may be explanatory for the decline in functioning of the immune system with age. With increasing age thymic involution leads a decreased output of naïve T cells, which subsequently leads to a reduction of peripheral T cell diversity, changes in

phenotype, altered cytokine production, modification in immune responses (25, 26). A decline in production of immune cells with increasing age is seen caused by changes in bone marrow constitution and decline in function of haematopoietic stem cells (27, 28). Another contributor to immunosenescence are the deficiencies in functioning of secondary lymphoid organs, causing less migration of immune cells to the spleen and therefore less antigenic stimulation (29). Both the innate and adaptive immune system appear to be affected by immunosenescence, where amongst many others deficiencies in numbers and inoptimal functioning of CD4+ T cells, CTL, B cells and NK cells are found (24, 30-32).

There has been increasing evidence that age associated immunosenescence might contribute to cancer development and progression (33). This raised the question whether age at diagnosis affects the interplay between the balance in cancer immune surveillance and tumor immune escape and consequently its effects on tumor progression and patient outcome in breast cancer patients. We priory determined tumor immune subtypes, which reflect tumor-immune interactions and represent a strong, validated, independent prognostic factor in breast cancer patients (22). We evaluated the distribution and prognostic effect of these tumor immune subtypes in elderly patients versus their younger counterparts.

MATERIALS AND METHODS

Patients and tumors

The patient population has been priory described in detail (22) and comprised all non-metastasized breast cancer patients primarily treated with surgery in the Leiden University Medical Center between 1985 and 1996. Patients with bilateral tumors or a prior history of cancer (other than basal cell carcinoma or cervical carcinoma *in situ*) were excluded. The following data were known: age, tumor grade, histological type, TNM stage, local and systemic therapy, locoregional/distant tumor recurrence, survival, and expression of estrogen receptor (ER), progesterone receptor (PgR), Ki67, and human epidermal growth factor receptor 2 (HER2) (34). All tumors were graded according to current pathological standards, by an experienced breast cancer pathologist. Approval was obtained from the Leiden University Medical Center Medical Ethics Committee. All samples were handled in a coded fashion, according to National ethical guidelines ("Code for Proper Secondary Use of Human Tissue", Dutch Federation of Medical Scientific Societies).

Immunohistochemistry and quantification of immunostaining

Immunohistochemistry and quantifications of immune markers used to construct tumor immune subtypes were previously performed as previously described in detail (13, 17,

35). Formalin-fixed paraffin-embedded tumor material was immunohistochemically stained according to standard protocols. Mouse antibody against CD8, PEN5 and Foxp3 were used for recognition of respectively CTL, NK cell and Treg infiltration (13, 35). Stainings for classical HLA class I were performed using the mouse monoclonal antibodies HCA2 and HC10 (13). Non-classical HLA class I molecules using mouse monoclonal antibodies against HLA-E and HLA-G (17).
Expression of classical HLA class I, combined HLA-E and –G expression, CTL infiltration, PEN5 infiltration and Treg infiltration were categorized respectively as loss versus expression, no expression versus expression, high versus low infiltration and absent infiltration versus present infiltration (35).

Tumor immune subtypes

Categorization of tumor immune subtypes, representing adaptive immune susceptibility of tumors was previously described (35). Briefly, different stages of immune surveillance and tumor immune escape were classified using combinations of CTL infiltration, NK-cell infiltration, Treg infiltration, classical HLA class I tumor expression and HLA-EG tumor expression. Tumors were first classified according to their immune susceptiblity resulting in the tumor immune subtypes which consisted of three clustered groups: "High immune susceptibility", "Intermediate immune susceptibility" and "Low immune susceptibility".

Statistical analysis

Statistical analyses were performed using the statistical packages SPSS (version 20.0 for Windows, Spps Inc, Chicago, IL, USA) and Stata (version 10.0 for Windows, StataCorp, College Station, TX, USA). The χ^2 test was used to evaluate associations between various clinicopathological parameters and tumor immune subtypes. Relapse-free period was defined as the time from date of surgery until an event (locoregional recurrence and/or a distant recurrence, whichever came first). Relapse-free period is reported as cumulative incidence function, after accounting for death as competing risk. The Kaplan–Meier method was used for survival plotting and log-rank test for comparison of relapse-free period curves. Cox proportional hazard analysis was used for univariate and multivariable analysis for relapse-free period. Relative survival was calculated by the Hakulinen method as the ratio of the survival observed among the cancer patients and the survival that would have been expected based on the corresponding (age, sex, and year) general population. National life tables were used to estimate expected survival. Relative excess risks of death were estimated using a multivariable generalized linear model with a Poisson distribution, based on collapsed relative survival data, using exact survival times. Analyses were stratified by age at diagnosis (<65 years versus ≥65 years). Multivariable analyses were adjusted for histological grade, histological type, T stage, N stage, estrogen receptor expression, progesterone receptor expression, HER2 status, local therapy and systemic therapy.

RESULTS

Patient and tumor characteristics

Of the total patient population (n=714), 469 (66%) were < 65 years at diagnosis and 245 (34%) were ≥ 65 years at diagnosis. Median age of patients was 58 years (range= 23-96 years). Clinicopathological and treatment characteristics of patients with available data for analysis are shown in Table 1. More detailed patient and tumor characteristics are described elsewhere (35).

Expression of immune markers by age

As priory described, immunohistochemical data of CTL infiltration, NK cell infiltration, Treg infiltration, classical HLA class I expression and HLA-EG expression were available for respectively 85% (607/714) and 91% (650/714), 95% (679/714), 83% (594/714) and 73% (519/714). Missing immunohistochemical data was due to tissue damage and unsuccessful staining of tumors. Cohen's kappa coefficient for inter-observer agreement of all these markers was determined previously and gave substantial to almost perfect agreements (13, 17, 35). The association between these markers and age is shown in Figure 1. Only HLA-EG expression showed a statistically significant inverse correlation with age (p=0.015), where expression of HLA-EG was more frequently found in patients aged <65 (27%) compared to patients aged ≥65 (17%). No statistically significant associations were found between patients aged ≥65 and patients aged <65 in frequency of high infiltration of CTL (31% versus 27%; p=0.253), present infiltration of PEN5 (51% versus 53%; p=0.599), present infiltration of Treg (45% versus 45%; p=0.991) or expression of classical HLA class I (77% versus 80%; p=0.282).

Tumor immune subtypes distribution by age

Tumor immune subtypes could be determined for patients with data available for all immune markers; 72% (512/714) of patients. Tumor immune subtypes showed the following distribution: "High immune susceptibility" 16% of patients (82/512), "Intermediate immune susceptibility" 67% (342/512), "Low immune susceptibility" 17% (88/512). Associations with known clinicopathological parameters are shown in Table 1. No statistically significant association was found between tumor immune subtypes and age (<65 versus ≥65; p=0.381), though low immune susceptible tumors were shown to occur more often in patients aged <65 (Figure 2).

Tumor immune subtypes and prognostic associations with outcome

The age-specific association of tumor immune subtypes with relapse-free period and relative survival are shown in Figure 3. In the group of patients aged <65 years, a strong association was found between immune subtypes and clinical outcome. Lower immune susceptibility, resulted in more relapses over time compared to higher immune susceptible tumors (RFP p<0.001, Figure 3 B; RS p< 0.001 Figure 3 E). Though a

similar trend was noticed in patients aged >=65 years, this was not statistically significant (RFP p= 0.147, Figure 3C; RS p=0.45 Figure 3F) Multivariable analyses were stratified for age and demonstrated that immune subtypes remained a statistically significant

	Total population analyzed		Immune susceptibility						
			High		Intermediate		Low		p-value
	N	%	N	%	N	%	N	%	
Age	341	67			222	65	64	73	0.381
<65	171	33	55	67	120	35	24	37	
>=65			27	33					
Grade									0.306
I	78	15	16	20	45	13	17	20	
II	246	49	33	41	173	51	40	47	
III	182	36	31	39	122	36	29	34	
Histological type									0.255
Ductal	460	91	71	89	314	92	75	87	
Lobular	46	9	9	11	26	8	11	13	
T-status									0.829
T1	192	38	31	38	131	39	30	36	
T2	242	48	42	52	159	48	41	49	
T3/4	66	13	8	10	45	13	13	16	
N-status									0.316
N0	267	54	48	60	178	54	41	48	
N1-3	231	46	32	40	155	46	44	52	
ER-status									0.093
Negative	205	40	39	49	138	41	28	32	
Positive	302	60	41	51	202	59	59	68	
PgR-status									0.460
Negative	231	46	41	51	155	46	35	42	
Positive	274	54	39	49	186	55	49	58	
Her2-status									0.352
Overexpression -	390	90	58	89	260	89	72	95	
Overexpression +	42	10	7	11	31	11	4	5	
Local Therapy									0.905
MAST-RT	207	40	34	42	139	41	34	39	
MAST+RT	99	19	17	21	62	18	20	23	
BCS	206	41	31	38	141	41	34	39	
Systemic therapy									0.622
CT alone	100	20	15	18	68	20	17	19	
ET alone	76	15	13	16	52	15	11	13	
CT&ET	21	4	1	1	18	5	2	2	
None	315	62	53	65	204	60	58	66	
Total	512	100	82	100	342	100	88	100	

Table 1 Correlations between molecular subtypes and well-established prognostic factors using chi-square test (missing data not shown).

Abbreviations N number of patients; % percentage; ER estrogen receptor; PR progesterone receptor; HER2 human epidermal growth factor receptor 2; MAST mastectomy; RT radiotherapy; BCS breast conservative surgery; ET endocrine therapy; CT chemotherapy.

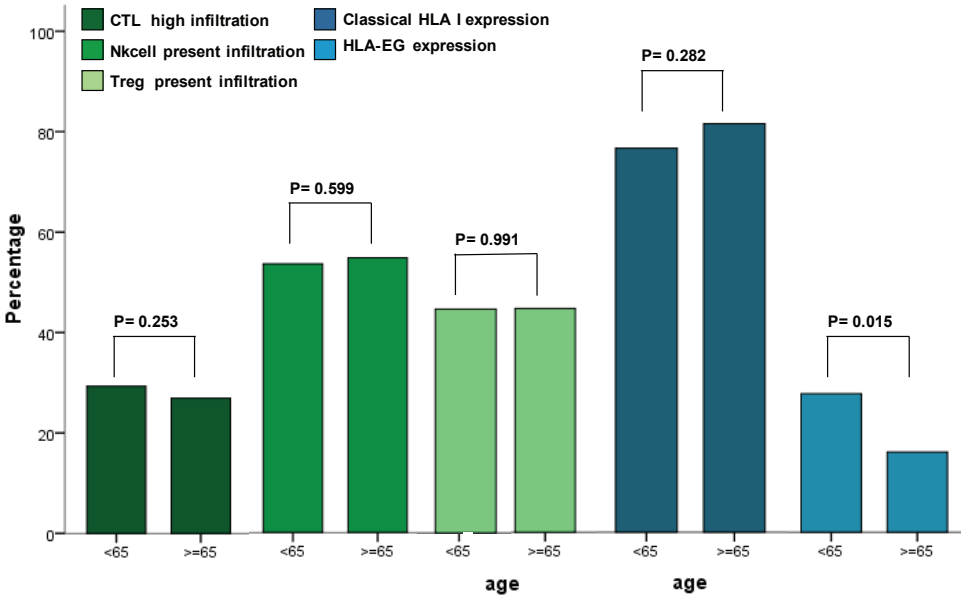

Figure 1 CTL, NK cell and Treg infiltration and classical HLA class I and HLA-E and HLA-G expression Histograms depicting distributions of high infiltration and high expression of these markers amongst age groups <65 and >=65 years are shown. Statistically significant less HLA-EG expression was found in patients aged >=65 years old.

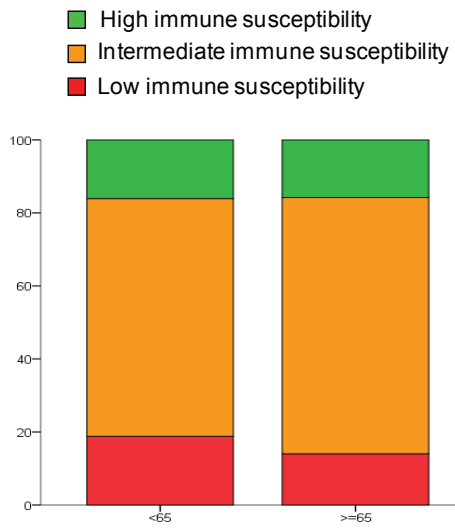

Figure 2 Tumor immune subtypes Bar chart depicting the distribution of immune subtypes according to patients aged <65 years and >=65 years. No statistically significant differences were found.

independent prognostic factor in young patients (RFP p<0.001, Table 2; RS p<0.001, Table 3). In patients aged ≥65 years, no statistical association was found in multivariable analyses between immune subtypes and clinical outcome (RFP p=0.15, Table 2; RS p= 0.45, Table 3).

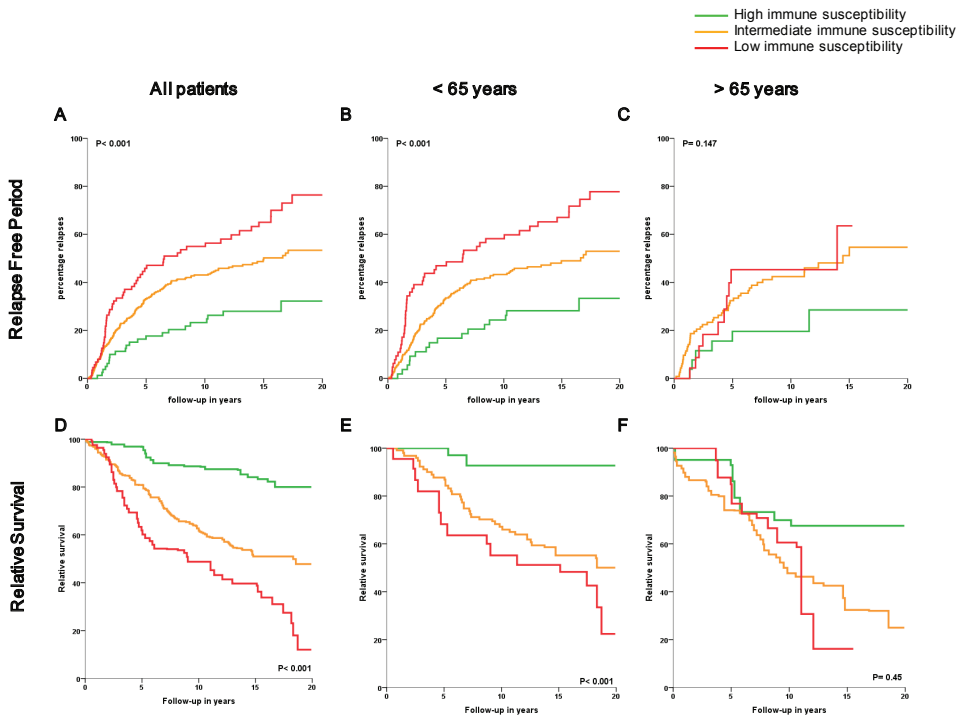

Figure 3 Kaplan Meier outcome analyses by tumor immune subtypes for Relapse free period (RFP) (A, B, C) and relative survival (RS) (D, E, F) according to the tumor immune subtypes. Tumor immune subtypes representing low immune susceptible resulted in a statistically significant unfavourable patient outcome concerning RFP and RS in patients aged <65 years. No statistically significant differences in outcome were seen in patients aged >=65 years. Log-rank P-values are shown in each graph.

DISCUSSION

In this study, we evaluated the distribution and impact on tumor progression and patient outcome of anti-tumor immune response and tumor immune evasion in elderly breast cancer patients compared to their younger counterparts. We compared previously determined numbers of infiltrating CTL, NK cells, Tregs, expression of classical HLA class I and HLA-E and –G and tumor immune subtypes, representing cancer immune susceptibility, between these two patient populations. Our results showed no differences in number of infiltrating CTL, NK cells or Treg, but a trend towards less classical HLA class I downregulation and statistically significant less HLA-E expression or HLA-G upregulation of tumors. These differences were also reflected, though not statistically significant, in less "low immune susceptible" tumors in patients aged >=65. Moreover, both RFP and RS outcome analyses showed tumor immune subtypes to be a statistically significant prognostic factor in young, but not in elderly breast cancer patients.

Characteristic		Patients < 65 years					
		Univariate analysis			Multivariable analysis		
	N	HR	95% CI	P	HR	95% CI	P
Grade							
I	74	1.00		0.03	1.00		0.40
II	225	1.32	0.86-2.02		1.24	0.66-2.34	
III	164	1.72	1.12-2.66		1.50	0.79-2.86	
Histological type							
Ductal	429	1.00		0.27	1.00		0.35
Other	36	1.31	0.82-2.10		1.37	0.71-2.63	
Tumor stage							
pT1	203	1.00		<0.001	1.00		0.13
pT2	210	1.53	1.14-2.05		1.38	0.89-2.12	
pT3/4	44	2.65	1.74-4.04		1.94	1.00-3.78	
Nodal stage							
Negative	249	1.00		<0.001	1.00		<0.001
Positive	213	2.85	2.16-3.76		3.46	2.18-5.50	
ER status							
Negative	216	1.00		0.61	1.00		0.44
Positive	236	0.93	0.71-1.22		0.85	0.56-1.29	
PgR status							
Negative	217	1.00		0.61	1.00		0.99
Positive	222	0.93	0.71-1.22		1.00	0.67-1.50	
HER2 status							
Negative	333	1.00		0.005	1.00		0.52
Positive	46	1.80	1.19-2.72		1.20	0.69-2.07	
Local Therapy							
MAST-RT	138	1.00		<0.001	1.00		0.56
MAST+RT	91	1.97	1.38-2.81		1.08	0.62-1.89	
BCS	24	0.78	0.56-1.07		0.84	0.54-1.29	
Systemic Therapy							
CT alone	120	1.00		0.037	1.00		0.003
ET alone	47	1.42	0.90-2.23		1.60	0.88-2.89	
CT&ET	25	0.74	0.38-1.44		0.37	0.14-0.98	
None	277	0.79	0.58-1.08		1.76	1.12-2.75	
Immune susceptibility							
High	55	1.00		<0.001	1.00		<0.001
Intermediate	222	1.93	1.14-3.26		2.77	1.46-5.24	
Low	64	3.51	1.98-6.19		4.61	2.32-9.18	

Table 2 Cox univariate and multivariable analyses for relapse free period stratified by patients aged <65 versus patients aged >=65 years.

Abbreviations N number of patients; HR hazard ratio; 95%CI 95% Confidence Interval; ER estrogen receptor; PR progesterone receptor; HER2 human epidermal growth factor receptor 2; MAST mastectomy; RT radiotherapy; BCS breast conservative surgery; ET endocrine therapy; CT chemotherapy.

Patients > 65 years						
	Univariate analysis			Multivariable analysis		
N	HR	95% CI	P	HR	95% CI	P
42	1.00		<0.001	1.00		0.019
117	1.84	0.89-3.80		1.93	0.63-5.97	
80	3.72	1.81-7.64		4.08	1.32-12.59	
209	1.00		0.49	1.00		0.19
30	1.23	0.69-2.22		2.17	0.68-6.90	
86	1.00		0.001	1.00		0.77
118	2.34	1.43-3.83		0.75	0.34-1.65	
33	2.68	1.35-5.32		0.78	0.25-2.47	
132	1.00		<0.001	1.00		0.004
100	3.18	2.06-4.89		2.76	1.39-5.49	
72	1.00		0.43	1.00		0.005
157	0.83	0.53-1.31		3.08	1.41-6.74	
99	1.00		0.18	1.00		0.02
129	0.75	0.49-1.14		0.43	0.22-0.85	
187	1.00		0.25	1.00		0.59
13	1.63	0.71-3.75		1.44	0.37-5.57	
147	1.00		<0.001	1.00		0.20
41	2.48	1.55-3.97		1.66	0.74-3.77	
57	0.49	0.27-0.90		0.56	0.22-1.41	
7	1.00		0.17	1.00		0.13
66	1.44	0.35-6.05		0.45	0.09-2.28	
2	3.09	0.43-21.99		4.77	0.34-67.55	
170	0.98	0.24-4.00		0.70	0.14-3.59	
27	1.00		0.16	1.00		0.15
120	2.24	0.96-5.23		2.62	0.98-7.01	
24	2.35	0.85-6.48		2.60	0.81-8.35	

Characteristic		Patients < 65 years					
		Univariate analysis			Multivariable analysis		
	N	RER	95% CI	P	RER	95% CI	P
Grade							
I	74	1.00		0.007	1.00		0.33
II	225	1.76	0.96-3.2		1.10	0.53-2.30	
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MAST+RT	91	1.84	1.25-2.71		1.12	0.59-2.11	
BCS	24	2.20	0.44-7.23		0.81	0.47-1.38	
Systemic Therapy							
CT alone	120	1.00		0.05	1.00		0.005
ET alone	47	1.54	0.93-2.54		1.82	0.91-3.62	
CT&ET	25	0.66	0.28-1.57		0.20	0.06-0.72	
None	277	0.84	0.59-1.20		1.46	0.87-2.43	
Immune susceptibility							
High	55	1.00		<0.001	1.00		0.001
Intermediate	222	2.55	1.22-5.31		3.93	1.68-9.18	
Low	64	4.01	1.24-8.74		5.53	2.28-13.40	

Table 3 Cox univariate and multivariable analyses for relative survival stratified by patients aged <65 versus patients aged >=65 years.

Abbreviations N number of patients; RER hazard ratio; 95%CI 95% Confidence Interval; ER estrogen receptor; PR progesterone receptor; HER2 human epidermal growth factor receptor 2; MAST mastectomy; RT radiotherapy; BCS breast conservative surgery; ET endocrine therapy; CT chemotherapy.

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30	1.39	0.62-3.09		1.18	0.18-7.87	
86	1.00		0.001	1.00		0.51
118	3.27	1.20-8.92		1.50	0.29-7.74	
33	8.73	3.08-24.77		2.69	0.46-15.8	
132	1.00		0.008	1.00		0.29
100	3.02	1.34-6.82		1.65	0.66-4.12	
72	1.00		0.027	1.00		0.57
157	0.45	0.22-0.91		0.64	0.14-2.99	
99	1.00		0.13	1.00		0.71
129	0.53	0.23-1.21		1.40	0.24-8.08	
187	1.00		0.02	1.00		0.07
13	3.10	1.24-7.76		3.19	0.91-11.2	
147	1.00		0.003	1.00		0.95
41	3.45	1.61-7.38		0.90	0.25-3.28	
57	2.53	1.01-1.95		0.80	0.18-3.59	
7	1.00		0.70	1.00		0.75
66	0.74	0.22-2.48		0.81	0.23-3.01	
2	0.74	0.05-10.01		0.79	0.10-11.2	
170	0.54	0.17-1.68		0.66	0.21-1.99	
27	1.00		0.45	1.00		0.45
120	2.85	0.46-17.74		3.57	0.42-30.3	
24	1.99	0.22-17.92		3.85	0.46-32.3 0.16-60.7	

The immune system plays an important role in the battle of the host against cancer development and progression (4). With aging, there are well-known alterations occurring in the immune response affecting both innate and adaptive immunity. It has been suggested that this process of immunosenescence might contribute to cancer development and progression, however this relation is nowadays still poorly understood (36). Previous studies have found differences in T cell and NK cell compartments between young and old people. T cells, especially CD8+ T cells, more often show poor proliferation, resistance to apoptosis and functional abnormalities, leading to a shift from so-called "truly naïve" T cells to "exhausted senescent" T cells. This reduced availability of naïve cells and T cell disfunctionalities are thought to explain the reduced ability of the elderly to respond to new antigens, including tumor associated antigens (37). NK cells also have shown to have decreased cytotoxicity and decreased IL-2 production in elderly patients (36). We found no statistically significant differences in number of infiltrating CTL or NK cells between breast cancer patients aged >=65 years versus aged <65 years. These results however do not contradict the theory of immunosenescence. As pointed out by previous studies, immunosenescence seems to be identified by a disfunctioning in immune recognition and cytotoxicity of CTL or NK cells, rather than by a non-capability of migration and infiltration in inflamed or carcinogeneous environments (36, 37). Like most retrospective immunohistochemical cohort studies, we were limited by the fact that we could not measure this direct functioning of tumor immune recognition and cytotoxicity. However, since data were present for both immune factors and tumor response, we were able to study interaction between the tumor and immune system, by which we could indirectly conclude on the function of the immune system.

During advancing oncogenesis, tumor immune recognition and attack by the immune system, causes immunoselection of target cancer cells, whom on their turn evolve variants able to resist immune attack. This results in the appearance of new tumor cells variants in order to maintain a state of equilibrium between the immune system and the tumor. The immune system must now exert new powerful selective pressures on the tumor cells, which will evolve again new variants able to resist this immune response, which finally leads to tumor immune escape (4, 5). It therefore is likely that a compromised immune system, as seen with aging, may lead to a left skewed shift in this tumor-immune equilibrium, where less tumor immune attack correlates with lower stages of tumor immune escape variant phenotypes (33, 36). Our results showed a statistically significant difference in HLA-EG upregulation and, though not statistically significant, a difference in classical HLA class I expression of tumors between elderly and younger breast cancer patients; less HLA class I downregulation and less HLA-EG upregulation were found in patients aged >=65 years. These results suggest that tumors in elderly patients have less need to downregulate expression of classical HLA class I and upregulate expression of HLA-EG, because less immune selective pressure is given by respectively CTL and NK cells. Our results strongly suggest a decreased

need for immune escape strategies in higher aged patients compared to their younger counterparts and are therefore in line with the left skewed tumor-immune equilibrium theory. Moreover, this theory is supported by the differences seen in distribution of tumor immune subtypes between patients aged <65 years compared to aged >=65 years; though not significant, tumors with low immune susceptibility are seen less in patients aged >=65 years.

Another method by which we indirectly measured the efficacy of tumor immune surveillance between elderly breast cancer patients and their younger counterparts were the associations of tumor immune subtypes with outcome. These subtypes were defined based on tumor susceptibility for cellular immune responses using expression of key factors in these responses: high CTL infiltration, presence of NK cells, and Tregs and tumor expression of classical HLA class I and HLA-E and -G. Outcome analyses of the immune subtypes in patients aged <65 years revealed strong associations with patient outcome where tumors defined as being highly susceptible to immune system attack showed a favourable outcome for breast cancer patients compared to patients with tumors defined as having a low immune susceptible profile. Though a trend towards similar outcomes was found in patients aged >=65 years, no such statistically significant association could be found. The fact that elderly breast cancer patients have comparable outcomes independently of the immune susceptibility of tumors is again highly suggestive for a less effective immune system in elderly patients and supports the hypothesis of immunosenescence and its contribution to cancer progression.

The phenomenon of immunosensence in humans is still hypothetical, but has previously been described in animal models where less immune responses were found after immunotherapy in old animals compared to young animals (38). The effects of immunosenesence on cancer development and progression have been suggested before, but to the best of our knowledge, we are the first to have found such age specific interactions between the immune system and cancer progression in a clinical dataset. These results might add to an explanation on the previously observed increase in breast cancer specific mortality with age by our group (3). While prior studies show tumors in elderly breast cancer patients to be of equal or of less malignant biological character than tumors found in their younger counterparts (39, 40), elderly breast cancer patients were in our study found to decease more often due to breast cancer regardless of a higher risk of mortality from other causes and independent of known tumor and patient characteristics. These contradictive findings might be explained by processes like immunosenescence and changes in tumor microenvironment, where it might not so much be the increased malignancy of tumor as the weakening of host defense against cancer determining tumor progression and therefore patient outcome. Future research is needed to confirm our results and to further unravel the complex interactions between immunosenescence, tumor progression and response to therapy. A better understanding

of these processes and future possibilities of immune manipulations and vaccinations might lead to more tailored treatment of elderly breast cancer patients.

In line with emerging evidence on immunosenescence in elderly and its hypothesized effects on tumor development and progression, we found less tumor immune escape variants and a fading prognostic effect of tumor immune subtypes in elderly breast cancer patients. To our knowledge we are the first to study the age-specific impact of the immune response and subsequent tumor immune evasion on tumor progression and patient outcome in a clinical set of breast cancer patients. Evidence based tailored treatment is highly necessitated in elderly breast cancer patients. Age-specific malfunctioning of the immune system in tumor control and it's implications on patient prognosis and response to treatments might aid in therapeutic decisions making for this specific breast cancer population. In addition, these data might contribute to the development of immune manipulations and cancer vaccinations.

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