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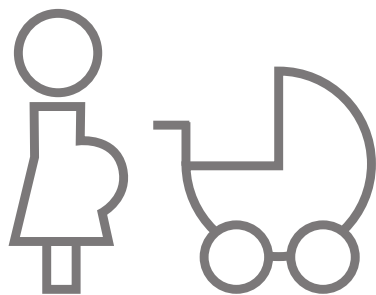
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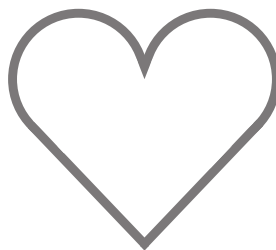
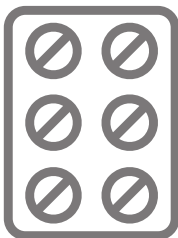


CHAPTER

4

Breast and colon cancer with
disorganized intra-tumor stroma and
hypertensive disorders in pregnancy
do not share pathogenesis of
abnormal cell to cell interaction

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ABSTRACT

Breast and colon cancer are leading causes of cancer-related death for women in the Netherlands. Intra-tumor stroma percentage (SP) has been described as strong parameter for survival for both cancer types. Pregnancy, specifically placentation, is also characterized by "tumor"-invasion: adequate placental invasion in the uterus is essential in favourable pregnancy outcome. Abnormal placentation may lead to pregnancy related complications such as preeclampsia (PE), recurrent miscarriages or intrauterine growth restriction.

We hypothesize that preeclampsia during pregnancy is an early clinical signal of decreased angiogenesis and is therefore associated with a low SP in women facing cancer later in life. We investigated whether women with signs of decreased angiogenesis (i.e. low SP) during breast or colon cancer are more prone to have a history of abnormal angiogenesis during pregnancy primarily characterized by PE.

Women included in databases for breast ($n = 208$) and colon ($n = 186$) cancer were obtained from the Leiden University Medical Centre. All included women received an invitation to fill out a validated questionnaire with respect to their pregnancy outcomes.

A total of 148 (52%) women were included in this study, 78 with a history of breast cancer and 70 with a history of colon cancer. Of women with a low SP ($n = 99$), 10.1% ($n = 10$) had a history of PE vs. 4.0% ($n = 49$) of women with high SP ($p = 0.17$). Women with a history of intrauterine growth restriction showed difference of 14.2% vs. 8.6%.

A history of PE was more often associated with low SP in both breast and colon cancer, although the differences across the SP categories with respect to PE did not reach significance. The data from this study suggest possible existence of parallels between disorders in placentation and development of tumors with low SP.

INTRODUCTION

Breast and colon cancer are leading causes of cancer-related death in the Netherlands and combined responsible for 17% of patients died due to disease in 2012 [1].

Metastasis is the leading cause of morbidity and mortality in colon and breast cancer patients [2]. Tumor invasion and metastasis are considered to be a multifactor process involving complex interactions of biological pathways [3]. Tumor-associated stroma invasion and cancer-associated fibroblasts play an important role in tumor progression [4]. It is hypothesized that processes similar to wound healing response are activated, which results in cell motility, angiogenesis, and matrix remodelling. For breast cancer, a wound-response gene expression signature, and a stromal signature have shown to be predictors of patient outcome and tumor progression [5-7]. These data suggest that stroma in the vicinity of tumors undergoes, or may be affected by, changes during tumor progression.

Recently, the intra-tumor stroma percentage (SP) within the primary tumor has been described as an independent parameter for survival for both colon and breast cancer. Patients with a high SP had a worse survival independent of tumor stage, tumor status and lymph node status compared to patients with a low SP [8-10].

Pregnancy is also characterized by “tumor”- invasion: adequate placenta invasion in the uterus is essential in good pregnancy outcome. Placentation is a process in early pregnancy defined by proliferation and differentiation of stromal cells into decidual cells, invasion of leucocytes, vascular remodelling and angiogenesis. In uncomplicated pregnancies uterine spiral arteries are invaded by endovascular trophoblasts and remodeled into dilated, inelastic tubes without maternal vasomotor control [11,12]. Preeclampsia, a pregnancy-specific complication, is characterized by defects in spiral artery remodeling and trophoblast invasion. The limited invasion of the spiral arteries results in reduced blood flow, which could eventually lead to clinically fetal intrauterine growth restriction (IUGR) and hypoxia [13].

Preeclampsia is a pregnancy unique disease defined as hypertension and proteinuria clinically manifested after 20 weeks of gestation [14]. Preeclampsia is the leading cause of maternal mortality and morbidity in the world as it is present in 5-7% of all pregnancies [15]. Other pregnancy complications described are to be related to impaired placentation are recurrent miscarriages and intrauterine growth restriction [16, 17].

HYPOTHESIS

Although, in a recent meta analysis no association between pregnancy complications (e.g. preeclampsia) and cancer was found, we hypothesize that preeclampsia during pregnancy is an early clinical signal of decreased angiogenesis and will result in a tendency to develop tumors with low stromal involvement in the tumor-microenvironment later in life.

The main goal of this study is to gain insight in the pathophysiology of breast and colon cancer by using pregnancy as a stress test: women who experienced a complicated pregnancy charac-

terized by abnormal angiogenesis may also have decreased angiogenesis in tumors developing later in life. Therefore, we assessed whether women with low SP tumors tended to have an obstetric history with more abnormalities related to uteroplacental dysfunction. The primary focus with respect to uteroplacental dysfunction was on preeclampsia, secondarily we assessed other abnormalities related to uteroplacental dysfunction like recurrent miscarriages and intrauterine growth restriction. This association and its potential pathway might create the opportunity for early detection in women's life of abnormal angiogenesis related cell-cell interaction.

EMPIRICAL DATA; MATERIALS AND METHODS

Study population

From the database of the Leiden University Medical Centre, a tertiary cancer centre in the city of Leiden, the Netherlands, we have selected a consecutive series of 206 female breast cancer patients who have been diagnosed between 1985 and 1994 at the LUMC [10]. The original database described by Kruijf et al consisted of 574 patients of whom 368 were known to be deceased at time of this study. Therefore 206 women could be included in this study. From the same database we have selected 337 female colon cancer patients who have been diagnosed between 1991 and 2001. Of these 337 patients 151 were known to be deceased, therefore 186 women could be included in this study.

Patient, tumor and treatment characteristics were retrieved from the original patient files of the database. For all patients, the haematoxylin-eosin (H&E) stained sections of the primary tumor were retrieved from the Department of Pathology.

For the breast cancer cohort, women were included with non-metastatic invasive breast cancer who were primary treated with surgery between 1985 and 1994 [10]. Patients with a history of cancer (other than basal cell carcinoma of the skin or in cervical intraepithelial neoplasia) were excluded. In the colon cancer group, women were included with non-metastatic colon cancer who had undergone complete potential curative treatment, including surgery alone or surgery plus radiation and/or chemotherapy between 1991 and 2001 [12, 13]. Histologically Duke B or Dukes C without gross or microscopical evidence of residual disease was reported.

All specimens were handled in a code fashion, according to national ethical guidelines ('Code for Proper Secondary use of Human Tissue', Dutch Federation of Medical Scientific Societies) [8-10, 18].

Intra- tumor Stroma Percentage

The intra-tumor stroma percentage (SP) was scored as described by Mesker et al. and the principles of this technique were applied in the evaluation of the SP in breast and colon cancer, as was reported previously [8-10]. In summary: routine tumor H&E stained slides from the primary tumor were used. Using a 5x objective the most stroma-abundant area within the tumor was localized and subsequently a 10x objective was used to estimate the percentage. Only image fields in which both tumor and stroma were present and in which tumor cells could be observed on all sides (north-east-south-west) were eligible for scoring. The percentage of tumor-associated

stroma was estimated in a tenfold manner and the highest stromal percentage was considered decisive. As described previously, stroma percentage $\leq 50\%$ and stroma percentage $> 50\%$ were categorized as stroma-low and stroma-high, respectively.

Questionnaire

All patients who were alive during the time of this study received an informed consent form and a validated questionnaire about their pregnancy outcomes. Six months after the first mailing, non-responders received a second mailing consisting of the informed consent form and the questionnaire.

The questionnaire is a validated self-administered questionnaire verifying the diagnosis of preeclampsia, eclampsia or toxemia on a group women with a greater than 20-year history of preeclampsia (**addendum 1**). Diehl et al [19] tested the accuracy of a questionnaire on preeclampsia. The questionnaire contained the same questions on preeclampsia previously described by Diehl et al. translated in Dutch. The questionnaire assessed whether one of the pregnancies was complicated by preeclampsia or intrauterine growth restriction and whether women experienced recurrent miscarriages. Intrauterine growth restriction was defined as a positive history on a child with a birth weight of 2500 grams or below. Recurrent miscarriages was defined as two or more miscarriages.

The institutional review board approved the protocol

Statistics

To obtain significant results in our study group with a power of 80%, alpha 0.05, and prevalence of preeclampsia 10% versus 4% as in our study group, we should include a total cohort of 283 women.

SPSS version 18.0 was used for data analysis. Dichotomous baseline data were expressed as numbers and percentages, continuous baseline characteristics were presented as medians and minimum and maximum values. For comparison of dichotomous follow up data of women with a history of preeclampsia or gestational hypertension at near term and women with a history of uncomplicated pregnancy, the chi-squared test was performed. For continuous data the one way Anova test was used. A p-value <0.05 indicated statistical significance.

EMPIRICAL DATA; RESULTS

208 women were eligible in the breast cancer group of which a total of 78 women with breast cancer were included. Reasons for exclusion: 17 women deceased, 91 women did not respond, 18 women did not experience a pregnancy or only had miscarriage(s) and 2 women did not fill in the questions on preeclampsia. In 2 additional cases, no SP was calculated.

186 women were eligible in the colon cancer group of which a total of 70 women with colon cancer were included. Reasons for exclusion: 24 women were deceased, 56 women did not respond, 20 women did not experience a pregnancy or only had miscarriage(s) and 4 women did not fill out the questions on preeclampsia. In 12 additional cases, no SP was calculated.

Figure 1 shows the inclusion process for this cohort study.

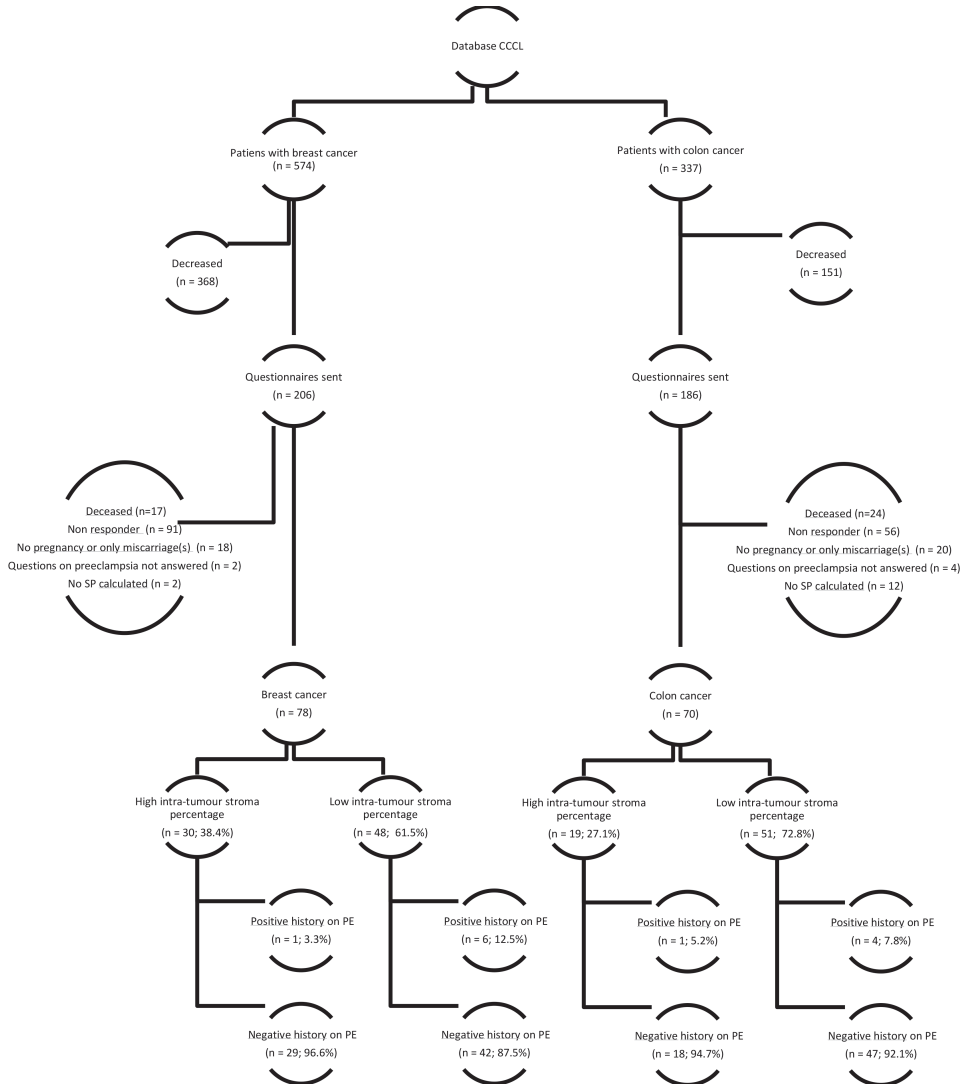


Figure 1.

Patient characteristics

Patient characteristics are displayed in **table 1**. Median age of women at the time of the primary tumor was 47.2 years in the breast cancer and 54.0 in the colon cancer group. In the breast cancer group most tumors were grade II, tumor stage T1 and nodal stage N0. In the colon cancer group most tumors were stage II, tumor stage T3 and nodal stage N0. There were no significant differences in age at primary tumor of diagnosis or tumor specifics between women with a high SP compared to women with a low SP.

Table 1. Patient Characteristics

	High SP	Low SP	Total
Breast cancer tumour	N = 30	N = 48	N = 78
Age (at time of primary tumour) <i>Yrs (min, max)</i>	48.3 (40 – 64)	46.2 (27 – 72)	47.2 (27 – 72)
Grade (<i>N (%)</i>)			
I	2 (6.9%)	7 (14.6%)	9 (11.7%)
II	19 (65.5%)	17 (35.4%)	36 (46.8%)
III	8 (27.6%)	24 (50.0%)	32 (41.6%)
Tumour stage (<i>N (%)</i>)			
T1	14 (46.7%)	23 (47.9%)	37 (47.4%)
T2	13 (43.3%)	18 (37.5%)	31 (39.7%)
T3	2 (6.7%)	6 (12.5%)	8 (10.3%)
Tx	1 (3.3%)	1 (2.1%)	2 (2.6%)
Nodal stage (<i>N (%)</i>)			
N0	22 (73.3%)	27 (56.2%)	49 (62.8%)
N1	7 (23.3%)	20 (41.7%)	27 (34.6%)
N3	1 (3.3%)	1 (2.1%)	2 (2.6%)
Colon cancer tumour	N = 19	N = 51	N = 70
Age (at time of primary tumour) <i>Yrs (min, max)</i>	49.8 (35 – 58)	54.3 (30 – 78)	54.0 (30 - 78)
Stage (<i>N (%)</i>)			
I	3 (15.8%)	17 (33.3%)	20 (28.6%)
II	8 (42.1%)	17 (33.3%)	25 (35.7%)
III	8 (42.1%)	15 (29.4%)	23 (32.9%)
IV	0 (0%)	2 (3.9%)	2 (2.9%)
Tumour stage (<i>N (%)</i>)			
T1	1 (5.3%)	7 (13.7%)	8 (11.4%)
T2	2 (10.5%)	13 (25.5%)	15 (21.4%)
T3	15 (78.9%)	27 (52.9%)	42 (60.0%)
T4	1 (5.3%)	4 (7.8%)	5 (7.1%)
Nodal stage (<i>N (%)</i>)			
N0	10 (52.6%)	34 (66.7%)	44 (62.9%)
N1	6 (31.6%)	12 (23.5%)	18 (25.7%)
N2	2 (10.5%)	3 (5.9%)	5 (7.1%)
Nx	1 (5.3%)	2 (3.9%)	3 (4.3%)

Table 2. Intra-tumour Stroma Percentage and Placenta related Pregnancy Complications

	Breast cancer			Colon cancer			Total		
	High SP	Low SP	<i>p</i>	High SP	Low SP	<i>p</i>	High SP	Low SP	<i>p</i>
	N = 30	N = 48		N = 19	N = 51		N = 49	N = 99	
History of preeclampsia	1 (3.3%)	6 (12.5%)	0.16	1 (5.2%)	4 (7.8%)	0.58	2 (4.0%)	10 (10.1%)	0.17
History of recurrent miscarriages	2 (6.6%)	3 (6.2%)	0.63	2 (10.5%)	3 (6.0%)	0.42	4 (8.1%)	6 (6.0%)	0.43
History of IUGR*	3 (11.1%)	8 (19.0%)	0.29	1 (5.2%)	4 (9.5%)	0.50	4 (8.6%)	12 (14.2%)	0.26

*IUGR = Intra Uterine Growth Restriction

Intra-tumor Stroma Percentage and Preeclampsia

In the total study group of women with colon and breast cancer, PE was 2.5 times more associated with low SP, however no significance was reached in history of PE between women with low SP and women with a high SP ($p = 0.17$). (**table 2**)

Of all women with a low SP ($n = 99$), 10.1% ($n = 10$) had a history of PE. Of all women with a high SP ($n = 49$), 4.0% ($n = 2$) reported a history of PE.

Of women with breast cancer and high SP ($n = 30$), 3.3% ($n = 1$) had a history of PE. Of women with low SP ($n = 48$), 12.5% ($n = 6$) had a history of PE ($p = 0.16$). (**table 2**)

Of women with colon cancer and high SP ($n = 19$), 5.2% ($n = 1$) had a history of PE. Of women with low SP ($n = 51$), 7.8% ($n = 4$) had a history of PE ($p = 0.58$). (**table 2**)

Intra-tumor Stroma Percentage and Recurrent Miscarriages and Intrauterine Growth Restriction

148 women reported on miscarriages. No significant difference in history of recurrent miscarriages between women with low SP and women with high SP was observed ($p = 0.43$). (**table 2**)

Of women with low SP ($n = 99$), 6.0% ($n = 6$) had a history of recurrent miscarriages. Of women with high SP ($n = 49$), 8.1% ($n = 4$) reported a history of recurrent miscarriages.

130 women reported on birth weight. No significant difference in history of intrauterine growth restriction between women with high SP and women with low SP was observed ($p = 0.26$). (**table 2**)

Of women with low SP ($n = 84$), 14.2% ($n = 12$) had a history of intrauterine growth restriction. Of women with high SP ($n = 46$), 8.6% ($n = 4$) reported a history of intrauterine growth restriction.

CONSEQUENCES OF HYPOTHESIS AND DISCUSSION

The hypothesis of shared pathogenesis of impaired placentation in preeclampsia and limited stroma invasion in tumor development can only be partly confirmed in this cohort study. This retrospective study showed no significantly higher percentage of a history of preeclampsia in women with low intra-tumor stroma percentage (SP) compared to women with high SP. However the trends observed in this study might indicate presence of shared pathways since the percentage of women with a low SP experienced more preeclampsia than women with a high SP (respectively 10.1% vs 4.6%).

Strengths and Limitations

To our knowledge, this is the first study that investigated the relation between two stroma-infiltrating disorders. Strength of this study is that it incorporates an important paradigm shift on tumor behaviour, which has gained substantial support during the past decade. When trying to understand tumor behavior we can no longer solely look at tumor cell specific characteristics. Instead, it is now widely accepted that traits of the micro-environment and of the patient also play an important part in tumor behaviour and prognosis [21]. The current study does both: assessed uteroplacental abnormalities in the patient and aimed to link this to micro-environmental characteristics (i.e. intra-tumor SP). Details on pathogenesis will subsequently be discussed.

Preeclampsia is a disorder of pregnancy with a high prevalence of 5-7% [15]. In our study group the prevalence was comparable with the general population with 8.1% of all cases. Although the prevalence is high, the fact that the absolute numbers of women with a positive history on preeclampsia in this study are low could contribute to the lack of significant findings in this study.

A limitation of this retrospective study is the sample size since we did not come towards the calculated sample size in this study.

For this study we used a questionnaire on the history of preeclampsia in women with breast or colon cancer. The overall response rate for this study was 60%, which could have caused recall bias. The questions on preeclampsia used in this study are validated to identify women with a greater than 20-year history of preeclampsia [19]. The validity of maternal-recall self-reported preeclampsia was further investigated by Coolman et al. [20] Conclusion of both studies showed a high specificity but low sensitivity i.e. moderate validity, which could cause bias in our study (80% sensitivity and 96% specificity in verifying a history of preeclampsia, with a PPV of 51%).

Shared pathways in Pathogenesis

Since the pathogenesis of placentation and oncogenesis are both very extensive and multifactorial, we chose to focus on the angiogenetic pathway. This choice is based on the assumption that in both preeclampsia and decreased stroma formation angiogenetic processes are of significant relevance.

An important hallmark in the development of tumors is angiogenesis. Hannahan et al described a model on the six biological capabilities acquired during the development of human tumors [21]. One of these is sustained angiogenesis since the oxygen and nutrients supplied by the vasculature are crucial for the cell function and survival within the tumor. Tumors appear to activate the angiogenic switch by changing the balance of angiogenic inducers, such as VEGF, and countervailing inhibitors. As stated above, the tumor-stroma invasion of cancer is known to correlate with prognosis in breast and colon cancer patients [11-13]. The micro-environment of the tumor, naming the stromal cells, contribute in high matter to the biological capabilities of the tumor [22]. Tumor angiogenesis is regulated by cancer cells expressing proangiogenic factors, further it is described that stromal cells are instrumental in switching on and sustaining chronic angiogenesis in many tumor types.

It is stated that the pathogenesis of preeclampsia consists of two stages. The first stage being poor placentation and the second stage is marked by activation of the systemic inflammatory process [23]. The first stage of poor placentation is caused by both oxidative and endoplasmic reticulum

stress of the placenta [24]. A state of hypoxia is therefore developed, causing an excessive production of soluble fms-like tyrosine kinase 1 (sFlt-1), which binds and deactivates circulating vascular endothelial growth factor (VEGF). An elevated level of sFlt-1 of maternal blood in mid-trimester pregnancy is known to predict preeclampsia [25]. Since deactivation of VEGF is attained, the important step in placentation of remodeling and angiogenesis of the spiral arteries is impaired.

In conclusion, although similarities can be found in the pathogenesis, this study showed a limited correlation in women with a history of preeclampsia and low intra-tumor stroma percentage tumors.

Addendum – Questionnaire verifying the diagnosis of preeclampsia

Did you ever had a pregnancy?

- a) Yes
- b) No

Did you ever had a miscarriage?

- a) Yes; how frequent? In what year?
- b) No

Did you had at least one pregnancy that lasted more than 6 months?

- a) Yes
- b) No

During any of these pregnancies (which lasted more than 6 months), did you have preeclampsia, eclampsia or toxemia?

- a) Yes
- b) No

During any of these pregnancies (which lasted more than 6 months), did you have hypertension / high blood pressure?

During any of the pregnancies in which you developed hypertension, did you have:

- a) Protein in the urine
- b) Seizures or convulsions
- c) Preeclampsia, eclampsia, or toxemia of pregnancy

Before the first pregnancy in which you developed hypertension, did you have:

- a) Protein in the urine
- b) Seizures or convulsions
- c) High blood pressure / Hypertension

Did you experience preeclampsia during your first pregnancy? Did you experience hypertension during your first pregnancy? (also for second, third etc pregnancies)

What was the term of pregnancies which lasted more than 6 months?

Of these pregnancies, what was the birth weight of your child / children?

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