Importance of physical trauma on recurrence of breast cancer

Can tissue trauma synchronize growth of dormant micrometastases?

Hanna Dillekås

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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Scientific environment

This work has been conducted at the Department of Clinical Science, Faculty of Medicine, University of Bergen. I have been affiliated with the CCBIO Research School for Cancer Studies. Main supervisor has been Professor Oddbjørn Straume, co-supervisors associate professor Svein Arthur Jensen and Professor Olav Mella.
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Abstract

Background

Surgical excision of the primary tumor is an essential part of breast cancer treatment. While breast cancer prognosis has improved dramatically over the past decades, late relapses after apparently successful primary treatment are still an unresolved clinical issue. Locoregional recurrences can be challenging and there is no cure in the case of distant metastases.

Purpose and aims

The general aim of the project was to evaluate the influence of tissue trauma and wound healing on metastatic relapse of breast cancer.

Methods

Detailed studies of relapse patterns and dynamics in several retrospective breast cancer patients' series were evaluated and related to surgical interventions as well as patients' factors like body mass index and perioperative events.

Results

A relapse pattern with multiple metastases of similar size was discovered in subgroups of patients, suggestive of a growth synchronizing event on dormant micrometastases. A peak in early relapses after delayed reconstructions supported the hypothesis that tissue trauma and wound healing may stimulate pre-existing occult tumor deposits. The effect seems to be different if surgery involves the removal of a tumor in the breast or not. Patients factors and perioperative events can modulate this effect.

Conclusions

This work demonstrates that tissue trauma and wound healing can have an impact on distant relapse dynamics in breast cancer. The mechanisms of this link remain to be fully elucidated in order to become targets for intervention.

List of Publications

This thesis is a summary of the following papers, referred to by their roman numerals in the text:

I. **Dillekås H**, Transeth M, Pilskog M, Assmus J, Straume O. Differences in metastatic patterns in relation to time between primary surgery and first relapse from breast cancer suggest synchronized growth of dormant micrometastases. *Breast Cancer Res Treat* (2014) 146:627-636

II. **Dillekås H**, Demicheli R, Ardoino I, Jensen SA, Biganzoli E, Straume O. The recurrence pattern following delayed breast reconstruction after mastectomy for breast cancer suggests a systemic effect of surgery on occult dormant micrometastases. *Breast Cancer Res Treat* (2016) 158:169-178

III. Demicheli R, **Dillekås H**, Straume O, Biganzoli E. Distant metastasis dynamics following subsequent surgeries after primary breast cancer removal. *Breast Cancer Research* (2019) 21:57

IV. **Dillekås H,** Demicheli R, Kristoffersen C, Jensen SA, Biganzoli E, Straume O. Perioperative factors and complications after delayed reconstruction in breast cancer patients in relation to oncologic outcome. *Manuscript*

Abbreviations

ATRA	All-trans retinoic acid
AZA	Azacitidine
BCT	Breast conserving therapy
BMI	Body mass index
BMP	Bone morphogenic protein
BRCA	Breast cancer associated gene
CBC	Contralateral breast cancer
CDK	Cyclin dependent kinase
CTCAE	Common terminology criteria for adverse events
CXCR	Chemokine receptor
DCIS	Ductal carcinoma in situ
DNA	Deoxyribo nucleic acid
EMT	Epithelial to mesenchymal transition
ER	Estrogen receptor
ERBB2	Erythroblastic leukemia viral oncogene homolog 2
ERK	Extracellular signal-regulated kinase
FGF	Fibroblast growth factor
GAS	Growth arrest specific
GFP	Green fluorescent protein

H&E	Haematoxylin and eosine
HER2	Human epithelial growth factor receptor 2
IBTR	Ipsilateral breast tumor recurrence
IFN	Interferon
IL	Interleukin
LCIS	Lobular carcinoma in situ
LPS	Lipopolysaccharide
MET	Mesenchymal to epithelial transition
miRNA	Micro ribonucleic acid
miRNA	Micro riboxy nucleic acid
MMP	Matrix metalloproteinase
NK	Natural killer
NR2F1	Nuclear receptor subfamily 2 group F
NSAID	Non-steroidal anti-inflammatory drugs
PDGF	Platelet derived growth factor
PGE2	Prostaglandin E2
PGR	Progesterone receptor
REC	Reconstructed
RECIST	Response evaluation criteria in solid tumors
ROS	Reactive oxygen species

SD	Standard deviation
TGF	Transforming growth factor
TNBC	Triple negative breast cancer
uPAR	Urokinase type plasminogen activator receptor
VEGF	Vascular endothelial growth factor
Zeb	Zinc finger E-box-binding homeobox 1

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1. Introduction

1.1 Breast cancer

1.1.1 Epidemiology

Breast cancer is the most frequent malignancy in women, globally causing 24,2% of all cancer and 15% of all cancer death in women, making it the cancer form responsible for most female cancer deaths worldwide (1). Breast cancer in men is a rare disease, although with a worse prognosis, presumably caused by a different biology as well as lower awareness of the disease leading to later diagnosis (2).

In Norway, median age at breast cancer diagnosis for women is 62 years, and the incidence is increasing. Some of this may be explained by improvement in diagnosis after introduction of mammography screening programs. However, the increase is also evident in women both below and above the age of inclusion in the screening program indicating other factors may be causing an actual increase in development of breast cancer. Risk factors include lifestyle aspects such as postmenopausal obesity (3), smoking (4), physical inactivity (5), low parity (6) and prolonged estrogen replacement therapy at menopause (7). Breast cancer survival has improved substantially over the past decades, reaching a 5-year relative survival rate of 90 %. Still, the risk of metastasis and breast cancer death remains after the 5-year time point, as demonstrated by a 15-year survival rate of 76 %. Manifest metastatic disease is considered incurable, and for stage IV disease, meaning distant metastases are present, 5-year survival is 29 %(8).

1.1.2 Diagnosis

Breast cancer is most commonly discovered by palpation of a lump in the breast or axilla or retraction of skin or nipple. Clinical findings suspicious of malignancy are evaluated with mammography and biopsy. Since the introduction of a mammography screening program and its expansion regarding age groups included, an increasing

proportion of breast cancer cases are detected before symptoms arise. The combination of increasing incidence and decreasing mortality has been attributed to earlier diagnosis, in combination with improved treatment. Criticism of this view has claimed that over half of screen detected breast cancers represent over-diagnosis of small tumors of unknown clinical importance (9). This has been refuted by others claiming there is a decline also in advanced breast cancer incidence. The Norwegian research council found in its evaluation of the mammography screening program a 30% reduction in breast cancer related mortality among participants in the program (10).

1.1.3 TNM-classification and staging

Breast cancer stage is determined by tumor size (T), lymph node status (N) and presence or absence of distant metastases (M) as outlined in table 1, and informs on prognosis and choice of therapy (11). Breast cancers are categorized as primary operable or primary inoperable according to staging, see table 2 (12). For the primary inoperable cases, unless distant metastases are present, down staging may be attempted by neoadjuvant treatment with chemotherapy or endocrine therapy, the purpose being to reduce tumor size and thus shift the stage to an operable stage, and thereby to improve prognosis.

Table 1. TNM Classification, 8th Edition

T0	No evidence of primary tumor
T1	≤2 cm (greatest dimension)
T2	>2-5 cm
T3	>5 cm
T4	Involving chest wall/skin
N0	No regional lymph node metastases
N1	Movable axillary lymph node metastases
N2	Fixated axillary lymph nodes or metastases to internal mammary nodes
N3	Supra- or infraclavicular lymph node metastases or combination of internal mammary and axillary lymph node metastases
M0	No distant metastases present
M1	Distant metastases present

Table 2. Operability according to stage level

Primary operable		Primary inoperable	
Stage I	T1N0M0	Stage II	T3N0M0
Stage II	T0-2N1M0	Stage III	T0-2N2M0
	T2N0M0		T3N1-2M0
			T4N0-2M0
			T0-4N3M0
		Stage IV	T1-4N0-3M1

1.2 Treatment

Treatment modalities for breast cancer consist of local therapy: surgery and radiotherapy, and systemic therapy: endocrine therapy, chemotherapy and targeted therapy. Treatment decisions are guided by anatomical considerations such as tumor size and lymph node involvement, molecular markers like estrogen receptor (ER), progesterone receptor (PGR) and human epithelial growth factor receptor 2 (HER2), and lately also genetic alterations such as BRCA mutational status (13). Gene expression profiles have recently been demonstrated to distinguish subgroups of patients with a very low risk of relapse where chemotherapy can be safely omitted (14, 15). Increasingly tailored treatment, targeting aberrant pathways according to molecular markers is rapidly evolving, exploring the weaknesses of the particular cancer of the individual patient.

1.2.1 Surgery

Surgical removal of the primary tumor can roughly be divided into mastectomy, where the entire breast is removed, and breast conserving surgery, meaning excision of the tumor with an additional margin, with most of the breast left intact. Both breast conserving surgery and mastectomy is accompanied by sentinel node biopsy, meaning excision of the breasts first draining lymph node. If this lymph node contains a metastasis >2 mm in diameter, axillary lymph node dissection has been recommended to all patients. Current recommendations allow for omitting lymph node dissection if certain criteria are fulfilled, such as planned systemic adjuvant treatment, even in the presence of a positive sentinel node. This approach is demonstrated to not increase risk of relapse (16), and reflects the shift in role of a positive sentinel node from a locoregional problem, best managed by local interventions to a biomarker of disseminated disease (17). Norwegian national guidelines state that surgical, oncological and cosmetic circumstances should be taken into consideration when planning surgery for breast cancer, with oncological principles given highest priority. Breast conserving surgery is in general preferred, and when combined with postoperative radiotherapy the procedure has not demonstrated inferior outcome compared to mastectomy (18, 19).

1.2.2 Radiotherapy

The purpose of postoperative radiotherapy is to reduce the risk of a locoregional relapse, but has also been demonstrated to improve disease specific survival. Thus, it must also indirectly reduce distant recurrences (20). Radiotherapy is recommended for all patients after breast conserving surgery, large or locally advanced primary tumor, lymph node positive disease or where clear surgical margins were not achieved (12). Radiotherapy is administered after chemotherapy when this is indicated, otherwise after the postoperative period.

1.2.3 Endocrine therapy

For estrogen receptor positive tumors, endocrine therapy can prevent systemic relapses when used in the adjuvant setting, and improve survival for patients with metastatic disease. Studies indicate clinical benefit when as little as 1% of tumor cells are positive for estrogen receptor, or 10% for progesterone receptor (21-23). Pre-menopausal women are recommended treatment with a selective estrogen receptor modulator, tamoxifen, for five years, for high risk patients combined with ovarian suppression with goserelin, thereafter evaluating menopausal status. If the patient remains premenopausal, and is considered in a medium to high risk group, prolonged tamoxifen for another five years has been demonstrated to further reduce recurrences and mortality (24). This illustrates the persistent risk of late relapse for this group of patients. For postmenopausal women, the primary choice is an aromatase inhibitor for five years (12).

1.2.4 Chemotherapy

The rationale behind systemic adjuvant chemotherapy is the risk that the cancer may be systemic at the time of diagnosis. Cytotoxic drugs target dividing cells. As cancer cells in general are rapidly dividing, they are susceptible to such drugs, as can be deducted by the one-third breast cancer mortality reduction compared to no chemotherapy (25). The benefit of this reduction in relative risk depends on the absolute risk, without chemotherapy, which must be taken into consideration as all chemotherapy regimens come with adverse side effects. Short term side effects include nausea, hair loss and immunosuppression, increasing attention is also given to long term adverse effects like fatigue, cognitive impairment, neuropathy and cardiovascular disease (26). As a consequence, more focus is given to de-escalating chemotherapy treatment as much as possible while maintaining oncologic outcome through better biomarkers and personalized treatment (27). The indication for adjuvant chemotherapy is determined by stage and expression status of hormonal- and HER2 receptors. For the low-risk patient group with ER+HER2-N0 status, it is

recommended to perform gene expression analyses to evaluate if chemotherapy can be safely omitted (27). When chemotherapy is indicated, it should in general include an anthracycline. In Norway, epirubicin, in combination with cyclophosphamide is given, for triple-negative and other high risk patients, followed by a taxane. Neoadjuvant chemotherapy may be used to reduce tumor size prior to surgery. Other advantages may be easier evaluation of treatment effect as the intact primary tumor may be evaluated for responsiveness. There has also been demonstrated a trend towards improved over all and disease free survival for preoperative compared to postoperative chemotherapy, possibly reflecting a benefit of early treatment of distant, undetected, micrometastases (28).

1.2.5 Targeted therapy

Targeted therapy is directed at defined molecular targets in the particular tumor of the individual patient. As such, endocrine therapy may be considered a form of targeted therapy as it is only given to patients with tumors expressing hormone receptors and these receptors are what the therapy targets. Still, by tradition, targeted therapy does not usually encompass endocrine therapy. An advantage of targeted therapies is that they, in general, come with milder side effects compared to chemotherapy. The first successful targeted therapy for breast cancer is usually ascribed to trastuzumab, a monoclonal antibody targeting HER2-receptors, preventing homodimerization and thus activation, with response demonstrated in the HER2 amplified subgroup of breast cancer (29). Introduction of cyclin dependent kinases (CDK)4/6-inhibitors is another example of successful targeted therapy for breast cancer. This group of compounds targets the transition from G1 to S-phase of the cell cycle, crucial for cell proliferation. In breast cancer, effect has been demonstrated in hormone receptor positive, HER2 negative metastatic disease in combination with endocrine therapy (30-32), and trials in the adjuvant setting are ongoing (NCT03078751, NCT02513394).

1.2.6 Immunotherapy

The dramatic responses to immunotherapy observed in tumor forms like melanoma and lung cancer (33) have, disappointingly, not been seen in breast cancer. So far, the small clinical benefit that has been reported is limited to the triple negative subtype, presumably because of a higher mutational load and elevated numbers of tumor infiltrating lymphocytes (34, 35). Trials further exploring a potential higher benefit by selection of patients, biomarkers, combinations with chemotherapy and timing of immunotherapy are ongoing (NCT03740893, NCT03395899, NCT03591276).

1.3 Reconstruction

The breast is an important part of the female body, regarding gender identity, self-confidence and sexuality. The strong wish for breast reconstruction in a large proportion of breast cancer treated patients is well acknowledged (36). Surgery is the corner stone of breast cancer treatment, but concerns have been raised regarding the oncological safety of breast reconstructive surgery. This has been evaluated in several studies with diverging results (37-39). Reconstructive techniques available today include relatively small surgical procedures such as implant based reconstruction, to more extensive flap-based reconstructions and bilateral correction procedures. Immediate reconstruction should, according to Norwegian guidelines be considered for all patients where mastectomy is indicated (12). Delayed reconstruction, one year after adjuvant local and systemic treatment is completed and no evidence of metastatic disease has emerged, was previously considered most appropriate, and is still preferred for some patients. Despite robust data demonstrating no survival benefit over breast conserving surgery (19, 40), mastectomy rates for early breast cancer are rising in the US (41). The reasons for this are not well understood but may be attributed to patients' overestimation of risk of recurrence, not sufficiently reciprocated by treating physicians. While mastectomy rates in Norway are decreasing (42), the need for plastic surgery to restore appearance after breast cancer treatment remains, although in a different form. Oncoplastic techniques, meaning

usage of plastic surgery techniques during cancer surgery to maintain shape and to some extent volume of the breast, are increasingly used. As a rule of thumb, breast conserving surgery involving removal of more than 15% of the breast volume is not considered likely to result in an acceptable cosmetic result unless oncoplastic techniques are applied (43).

1.4 Tumor biology and the hallmarks of cancer

Cancer is characterized by loss of normal control mechanisms regulating proliferation and homeostasis, transforming a normal cell into a cancer cell. The complexity of these control systems is mirrored in the multifaceted alterations present in cancer. In 2000, Hanahan and Weinberg published a review article describing a set of functional capabilities, or hallmarks, shared by most, if not all cancers, rationalizing this complexity (44). These were: self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis. These capabilities are acquired by gain of function mutations in oncogenes and loss of function mutations in tumor suppressor genes, caused by insertions, deletions, genomic rearrangements, copy number alterations and epigenetic modifications. To these hallmarks two emerging hallmarks were later added: deregulating cellular energetics and avoiding immune destruction. Additionally, two enabling characteristics: 1) genome instability and mutation and 2) tumor promoting inflammation were included (45). This represents a move from an important, but reductionist focus of cancer as a disease of the genome, to an understanding of tumors as complex tissues with aberrations also in stroma and non-cancerous cells.

1.5 Tumor heterogeneity

Breast cancer is a heterogeneous group of malignancies with variable genetic characteristics resulting in different prognoses and responses to therapy. The classic

subgrouping according to hormone receptor status and HER2 amplification status guides treatment decisions as these markers are predictive of response to antiestrogen and anti-HER2 directed therapy respectively. They also inform of prognosis and relapse dynamics. The triple negative breast cancers, meaning cancer cells lacking expression of estrogen and progesterone receptors and not displaying HER2 amplification, have the worst prognosis (46) whereas the estrogen receptor positive cancers have the most favorable outcome (47).

The seminal publication on breast cancer subtyping based on gene expression profiles by Sørlie et al divided breast cancer into five distinct groups with different outcome, luminal A, luminal B, normal breast like, ERBB2+ and basal like (48). These groups somewhat, although not completely, overlap with traditional cell surface markers. The luminal A and B being ER positive and HER2 negative, the basal like being mainly triple negative and the ERBB2+ being HER2 enriched. The tumor microenvironment is also diverse in the different types of breast cancer. One example is the higher levels of immune cell infiltration in triple negative cancer, suggestive of a higher likelihood of effect of immunotherapy (49). While these are examples of inter-patient heterogeneity, meaning differences between patients, breast cancer also demonstrates intra-patient heterogeneity referring to differences between tumor manifestations within the same patient. Whole genome sequencing has demonstrated significant variation in alterations and mutations between the primary tumor and its metastases, with most metastases having acquired additional driver mutations not present in the primary tumor. This may be a result of both selective pressure from therapy and the immune system, as well as continued acquisition of mutations as an effect of deficient DNA-repair mechanisms (50).

1.6 Relapse patterns

The relapse patterns vary with breast cancer subgroup. The triple negative, basal like, tumors have the highest risk of relapse the first five years after primary treatment and the estrogen receptor positive ones, particularly the luminal A subgroup the lowest

(51). The risks, however, are time dependent, meaning that while the risk of relapse declines for the high-risk tumors after the first years, the risk is stable for a subgroup of the low risk tumors (52, 53), with relapses occurring as late as 25 years after apparently successful primary treatment. In addition to tumor characteristics, patient factors like overweight and obesity have been demonstrated to influence the relapse dynamics in breast cancer (54). A bimodal relapse pattern of breast cancer after primary therapy has been demonstrated in multiple patient series, with a first peak in relapses after roughly two years, and a second one at five to six years (Fig 1) (54-56).

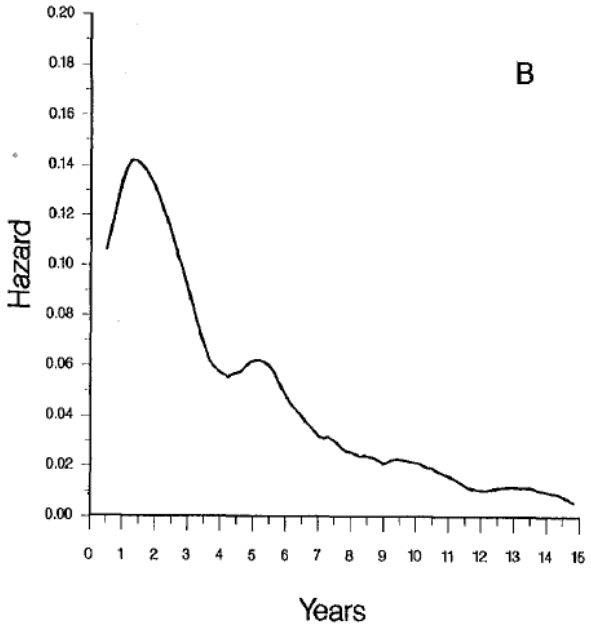

Fig. 1 First demonstration of the bimodal relapse pattern in breast cancer, from the Milan series, n=1173. Reprinted from (55) with permission.

The first peak is, although not uncontroversially, explained by some to be elicited by a stimulating effect of primary tumor surgery, while the latter is considered to be the result of an accumulation of stochastic mutational events (57). Metastasis

organotropism is the tendency for a given cancer form to metastasize to certain organs. Breast cancer preferentially metastasizes to bone, liver, lungs and brain, with some variation between subtypes (58). While locoregional relapses may be diagnosed at routine follow up, distant metastases are usually detected when they cause symptoms, as follow up in Norway does not include imaging or blood sampling to detect biochemical alterations. It has repeatedly been demonstrated that physical examination and mammography are as effective as extensive follow up regimens regarding recurrence detection, overall survival and quality of life (59). As speed of metastatic development is heterogeneous, and patients vary in their proneness to seek medical advice when experiencing symptoms, distant metastases may have been present for a short or long time when diagnosed.

1.7 The metastatic process

Metastatic cancer is, with very few exceptions, incurable, and a majority of cancer-associated deaths can be attributed to metastatic disease (60). Understanding the processes leading from localized to systemic cancer disease is therefore of utmost importance. In order to metastasize, cancer cells must undergo a series of events, all of which are, to a varying degree of success, attempted counteracted by the host (61).

The first step in the metastatic cascade is detachment of cancer cells from the primary tumor and invasion into surrounding tissue matrix (62). This requires a reprogramming of the cells, from an epithelial state into a more mesenchymal, and thereby migratory phenotype. This process has been termed EMT, epithelial-to-mesenchymal transition (63, 64) and will be expanded on in the subsequent section on wound healing. The invasive cells must then penetrate the blood or lymphatic vessel walls to intravasate to be transported to distant organ sites (65). In the blood stream, the cancer cells will encounter a range of host immune cells, which, depending on the degree of neoantigen expression and immune regulatory surface proteins on the cancer cell, may recognize it as foreign and elicit an immune response (66). In addition, the physical conditions with extensive shear stress must be

overcome (67). Other constituents of the intravasal environment may be beneficial to cancer cell survival. Platelets have been suggested to protect circulating tumor cells both by physically masking them with fibrinogen and by secreting factors such as transforming growth factor β (TGF-β) and platelet derived growth factor (PDGF) that inhibit anti-tumor immune activity (68).

Upon arrival at a suitable metastatic niche, the cancer cell must extravasate and lodge in the pericapillary regions of the target organ. This process will most likely be very heterogeneous depending on the target organ, where bone marrow and liver with fenestrated capillaries, will permit passive diffusion of cancer cells. At the other end of the scale we find the blood-brain barrier needed to be penetrated in order to establish metastases in the central nervous system. After having succeeded with extravasation, the cells must undergo a mesenchymal-to-epithelial transition to regain proliferative traits needed to colonize the target organ (69). The metastatic process is documented to be highly ineffective, experimental evidence suggests that as few as 0,02% of circulating tumor cells are able to form macrometastases despite the fact that the early steps are highly efficient (70). As early as in the 1880's, Stephen Paget launched his "seed and soil"-theory, stating that in order for cancer to metastasize, the best suited cancer cell (seed) needs to find, and interact with, a receptive microenvironment (soil), this theory, to a large extent still holds forth today (71).

1.8 Tumor dormancy

1.8.1 Definitions and mechanisms

Tumor dormancy, first described in 1954 (72), refers to a reversible state of little to no growth of cancer cells with maintained malignant potential and can be divided along several different axes. Primary tumor dormancy, where people harbor microscopic tumors without ever having been diagnosed with cancer, has been demonstrated in autopsy studies to be very frequent (73). Metastatic dormancy on the other hand is characterized by a latency to manifest metastatic disease after

apparently successful primary tumor treatment, thus resulting from early dissemination and a period of dormancy at the metastatic site. This has been ascribed to a maladaptation to the new microenvironment of the metastatic site, which may be quite different from the organ of origin, not permitting growth of the cancer cell until it has acquired new characteristics, better suited to this new milieu, or the microenvironment changes (74). Cancer treatment with chemotherapy can also induce dormancy, as has been demonstrated *in vivo* to be mediated by type I interferon (IFN) signaling. A clinical correlate in human was suggested by an association between serum IFN-β during neoadjuvant chemotherapy and longer time to recurrence (75). Withdrawal of hormonal stimulation can maintain cancer in the dormant state, as can be deducted from both the benefit of extended endocrine therapy, even after 5 years (24, 76), and by the synchronization of metastasis growth at ended adjuvant endocrine treatment, presented by us in this current work (77). In an ovariectomized immunocompromised mouse model, ER+ breast cancer micrometastases remained dormant until hormone therapy was initiated (78).

The distinction can also be made between intrinsic dormancy, caused by genetic or epigenetic mechanisms within the cancer cell (79), and extrinsic dormancy, where micrometastases are kept dormant by immune control (80), angiogenesis restriction (81) or growth factor deprivation (82). Partly overlapping with the classification of intrinsic and extrinsic dormancy is the concept of cellular dormancy, cell cycle arrest in G0 phase of individual cells and population-based dormancy where there is a balance between proliferation and apoptosis (83). These classifications or variants of tumor dormancy are not mutually exclusive and can thus exist in the same patient at the same time.

1.8.2 Clinical evidence of dormancy

The fact that some cancer forms, breast cancer being perhaps the most widely recognized, can give rise to metastatic disease years or decades after primary treatment is considered indirect evidence of a period of dormancy as this cannot be

convincingly explained by other models of tumor growth (55). Evidence for immune mediated dormancy stems from the occurrence of donor derived cancer, where organ recipients, under immune suppressive treatment, develop cancer originating from donors considered cured from cancer, most frequently melanoma (84) but also other cancer forms, including breast (85). The cancer-immune system interaction is considered to span a scale of escape, equilibrium and elimination (86). Most cells undergoing malignant transformation are recognized and eliminated by the immune system. Some are able to escape the immune killing and cause clinical cancer. Micrometastatic dormancy is proposed to exist in a state of equilibrium with the immune system, where the micrometastatic deposit is prevented from expanding but able to survive.

1.8.3 Biomarkers of dormancy

A marker for the presence or absence of disseminated dormant cancer cells would be important information for follow-up of cancer patients. As of now, there is no clinically validated biomarker of dormant cancer. In breast cancer, the presence of disseminated tumor cells in the bone marrow after primary treatment is an independent prognostic marker (87), but we are not yet capable of distinguishing if these cells are truly dormant and harbor the potential of awakening. From basic science, the nuclear receptor NR2F1 displays potential as a marker of dormancy, and is currently being explored in clinical samples in breast cancer as well as other tumor forms (88, 89). Liquid biopsies are significantly easier and less invasive than bone marrow aspiration and have also demonstrated usefulness as a prognostic biomarker of late recurrence. In a series of ER positive, HER2 negative patients, without evidence of recurrence five years after primary treatment, detection of circulating tumor cells at five years was significantly associated with relapse (90). In a small group of late relapsing breast cancer patients, significantly differential expression of miRNA-21 and miRNA-200c was discovered in plasma at primary treatment compared to non-relapsing patients (91). TGF-β2 (92), bone morphogenic protein

(BMP) (93), growth arrest specific (GAS6) (94), retinoic acid, and IFN-β (75) are other systemic markers explored for potential clinical utility as biomarkers for tumor dormancy.

1.9 Wound healing and cancer as a wound

The process of wound healing is a carefully orchestrated series of events involving multiple local and systemic changes, all with the intent to restore tissue homeostasis, regain function and protect from infection. Whether the wound is caused by a traumatic event or deliberately inflicted by surgery, successful healing is crucial, as demonstrated by the major health issues caused by deficient wound healing capacity seen in diabetic patients amongst others. The analogy of cancer as "wounds that do not heal" was first presented by Harold Dvorak in 1986, mainly relating to the similarity of cancer stroma to granulation tissue (95). As described below, and previously reviewed by us (96), this metaphor holds true also when examining the wound healing phases and signaling today.

1.9.1 The immediate response

The first phase, initiated at tissue trauma, is blood clotting, if the injury involves blood vessels. The blood clot, predominantly consisting of cross-linked fibrin and platelets, previously considered merely a temporary physical sealing of the wound, is now known to have several biological functions such as release of growth factors from platelet granules and induction of vascular permeability by histamine and VEGF (97). VEGF also has immunosuppressive effects, potentially releasing cancer cells from immune restriction (98). Fig 2a. This, together with complement activation rapidly leads to the next phase, inflammation by chemoattraction of inflammatory cells. The cocktail of growth factors and other cytokines released by platelets upon activation has the potential to promote proliferation and migration of cancer cells (68).

1.9.2 Inflammation

First to arrive are the neutrophils, followed by macrophages and lymphocytes. Neutrophils secrete prostaglandin E2 (PGE2), and reactive oxygen species (ROS), further fueling inflammation with the purpose of eradicating invading bacteria (99). Macrophages also secrete PGE2 when stimulated by pro-inflammatory cytokines such as IL-6, and bacterial lipopolysaccharides (LPS)(100). PGE2 is prominent among the inflammatory mediators that also exert tumor-sustaining effects (101, 102). Fig 2b. Among its functions in wound healing, that can also be utilized by cancer, are stimulation of proliferation, migration and angiogenesis, leading on to the next phase.

1.9.3 Proliferation, re-epithelialization and contraction.

Keratinocytes and fibroblasts from the wound margins are stimulated to migrate into the wound and proliferate, mainly by TGF-β which is secreted from activated platelets in the early phase and later by several cell types (103). In keratinocytes, this is preceded by a partial epithelial-to-mesenchymal-transition, EMT, whereby the keratinocytes downregulate cell-to-cell contact and hemidesmosomes, rearrange the cytoskeleton, extend lamellipodia and secrete proteases to be able to degrade connective tissue and move into the granulation tissue of the wound (104). Again, these mechanisms, when applied to cancer cells, facilitate invasion and dissemination and thus metastatic seeding. Fig 2c. In cancer, the EMT is more frequently complete, characterized by complete loss of cell-to-cell adhesions and expression of mesenchymal marker proteins like vimentin, resembling early embryogenesis, as opposed to the partial EMT in wound healing keratinocytes (64). In physiological wound healing, the keratinocytes, by an as of yet undetermined signal, reverts to the epithelial phenotype at completion of wound healing. For cancer cells to regain their proliferative potential, a mesenchymal-to-epithelial transition, MET, is also necessary

as the decision between EMT/MET state determines if the cell should “go or grow”. In wound healing, this MET takes place at the final phase, resolution and remodeling (103).

1.9.4 Resolution and remodeling

Resolution and remodeling is the least well understood and mapped phase of wound healing. Evidence of the importance of an ordered end of inflammation, proliferation and scar tissue deposition is seen both in chronic wounds and development of keloid scars where this final phase is hampered (105). Wnt signaling initiates epidermal development programs to re-stratify keratinocytes, this phase also includes regrowth of appendages (106), MMPs degrades and remodels the extracellular matrix to form organized collagen and proteoglycans, neutrophils and macrophages undergo apoptosis or return to the vasculature and inflammation resolves. Fig 2d.

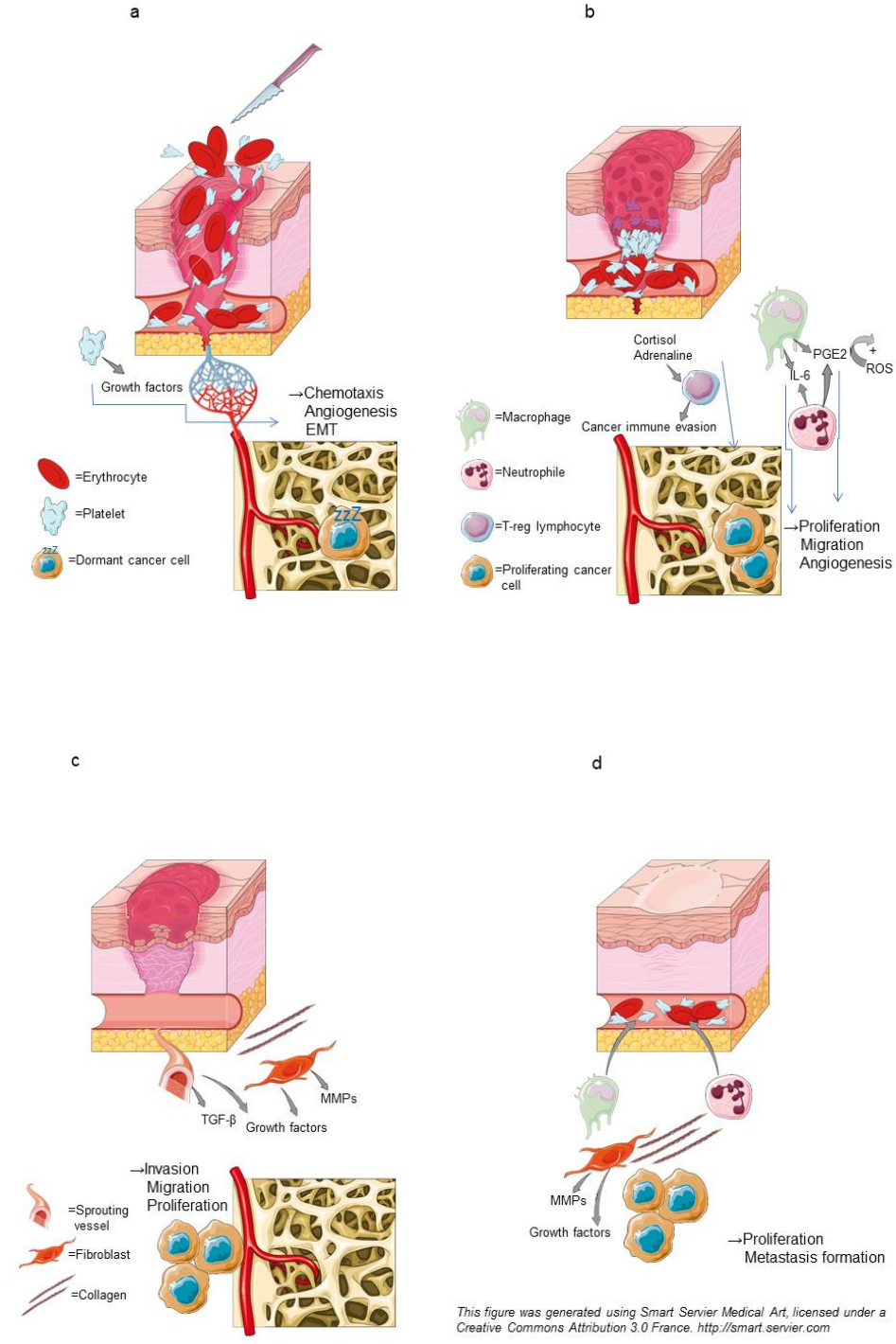

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Fig. 2 a) The immediate response. Activated platelets release growth factors that recruit inflammatory cells and stimulate vessel sprouting, re-epithelialization and degradation of matrix, but may also stimulate pre-existing dormant tumor cells at a distant site to proliferate and migrate. b) Inflammation. Neutrophils and macrophages secrete growth factors, cytokines, reactive oxygen species (ROS) and prostaglandin E2 (PGE2), fueling an inflammatory response in the wound and surrounding tissues. Systemic levels increase, and these factors are also known to be able to stimulate proliferation and migration of tumor cells. Systemic release of cortisol and adrenaline stimulates T-regulatory cells and can thus aid in cancer immune evasion. c) Proliferation, migration and contraction. Sprouting vessels produce tumor-promoting factors such as TGF-β. In order to re-epithelialize the wound surface, keratinocytes undergo a partial EMT. Fibroblasts generate scar-tissue and can aid cancer cells in invasion and migration. d) Resolution and remodeling. In normal tissue, inflammation and proliferation resolves by unknown mechanisms when tissue is regenerated, inflammatory cells return to the vasculature. A stiff, fibrotic environment can determine cytoskeletal reorganization inducing proliferation and metastasis formation in cancer cells located in this environment. Adapted from (96), with permission.

1.10 Tissue trauma and cancer

Surgical removal of the tumor was one of the first successful approaches to treat cancer, and to this day remains the cornerstone in curative treatment of many primary tumors. Breast cancer, however, is a clear example of the limitations of surgery. The repeated relapse of breast cancer, after successful removal of the primary tumor, led to increasingly aggressive surgical procedures. The most dramatic example being the mutilating ultra-radical mastectomies by Halstead in the late 1800s, based on the idea that relapse would be avoided by taking the tumor by its roots. Systematic follow-up of these patients demonstrated the futility of this practice as no improvement in distant relapse-free survival was detected after these very extensive surgeries (107).

The fact that the cancer could relapse, regardless of the extent of surgery, inspired the idea that dissemination to distant sites could be by the hematogenous route and thus independent of the anatomic limitations of the breast and associated lymph nodes. In an impressive effort to determine what physiological signaling could stimulate growth of metastases, the Fisher brothers, in the 1950's and 60's, performed a series of animal experiments testing a vast array of different interventions in rats implanted with a mammary carcinosarcoma. The stimuli ranged from anticoagulants and nutrition to the pituitary gland, thyroid and importantly, surgical trauma, which resulted in an increase in liver metastases after both laparotomy and liver resection (108, 109).

When considering the events that must take place in order for a wound to heal, as outlined above, it is almost intuitive that cancer can benefit from the involved signaling: cells must proliferate and migrate, blood vessels must be made more permeable for cells to enter into and exit from the bloodstream. Add to that tumor fueling inflammation, an inevitable part of wound healing and the stage seems set for tumor growth and metastasis (45). Ample preclinical and clinical evidence of such an effect in many cancer forms has been published (110-113). It must be mentioned, however, that the exact opposite effect, namely inhibition of tumor growth by surgical trauma in adjacent tissue, has also been reported from animal models. In this study, the growth restrictive effect after repeated full skin excision was attributed to competition for growth factors (114). The survival benefit of surgically removing the primary tumor is undisputable and will remain an important part of cancer treatment in the foreseeable future. The systemic biological response to surgery, however, may be better understood and harnessed in order to minimize undesired cancer stimulating effects.

1.11 Perioperative interventions

As outlined above, the biology of tissue trauma and wound healing is complex and may have significant systemic effects. In addition, a range of systemic interventions

are made in the perioperative period to improve short term outcome and perioperative morbidity and mortality. This includes good hemostatic technique to reduce bleeding, prevention of infection and thromboembolism. To make large surgical procedures possible, anesthesia and analgesia are also necessary. Both the tissue trauma of surgery per se and the associated systemic interventions may have long term impact on oncologic outcome, and are attracting increasing attention (115, 116). Most reports on effects on oncologic outcome of perioperative interventions are based on retrospective analyses, thus the risk of a bias in selection of patients for the considered intervention is clearly present.

From retrospective studies, intravenous anesthesia with propofol seems to improve oncologic outcome compared to inhalation anesthesia with sevoflurane, in breast cancer (117) and other cancer forms (118, 119). In a prospective randomized controlled trial of breast cancer surgery however, no statistically significant difference in 2-year relapse free survival was detected (120). A large, randomized study comparing short- and long-term survival between propofol and sevoflurane at primary surgery is currently recruiting patients with breast, colon or rectal cancer (NCT01975064).

Treatment with NSAIDS (non-steroidal anti-inflammatory drugs) in the perioperative period has been suggested to have a beneficial effect on oncologic outcome. This has been supported by retrospective clinical data (121, 122) and pre-clinical studies (123, 124), but again failed to demonstrate improved disease-free survival in a prospective trial of high risk breast cancer patients (125).

The risk of thromboembolism is elevated both after surgery and the often accompanying immobilization and in cancer patients in general (126). Perioperatively, low molecular weight heparin is administered when the risk of thromboembolism is considered elevated, by patient factors such as obesity, cardiovascular comorbidity, smoking and active malignant disease, or surgery factors such as long duration of procedures. Epidemiological studies have suggested a survival benefit to cancer patients treated with low molecular weight heparin beyond

prevention of thrombosis, although results are inconsistent (127). Experimental systems have demonstrated anti-cancer properties of this class of drugs such as reduced sphere formation, migration, invasion, and angiogenesis (128, 129). A randomized controlled trial has demonstrated a survival benefit of a short course of low molecular weight heparin in patients with metastatic cancer, although no significant effect was seen in the breast cancer subgroup (130).

1.12 Escape from dormancy

Stimulated growth of cancer lesions after tissue trauma and wound healing has been demonstrated repeatedly, both in clinical and experimental settings (110, 112, 113). As mechanisms of dormancy are not yet fully understood, the evidence for how tissue trauma and wound healing can facilitate escape from dormancy is less clear.

Coherent with the concepts of extrinsic and population-based dormancy is the theory of an angiogenic switch being capable of inducing escape from dormancy. This was first proposed by dr Judah Folkman in the 1970's. According to his work, tumors of 1-2 mm in size are restricted from further growth by lack of sufficient blood supply (131). At some time point, the hypoxia resulting from this hypoperfusion stimulates production of angiogenic factors such as VEGF, FGF, angiopoietins and others, causing blood vessels to sprout and supply the tumor with oxygen and nutrients to support further growth (132). This switch, from a non-angiogenic to angiogenic phenotype mediating escape from dormancy has been demonstrated in animal models, but as of yet lacks a convincing clinical correlate (133, 134). Another angle of the vascular-dormancy interaction is the proposal of the perivascular niche of stable vessel as dormancy inducing and maintaining via enrichment of thrombospondin-1, while tip cells of sprouting neovasculature produce periostin and TGF-β, promoting metastatic growth (135).

The primary tumor is suggested to be capable of maintaining microscopic metastases in a dormant state, in models demonstrated to be mediated by production of

angiogenesis inhibitors (136) or immune modulation by IL-1β (137). Accordingly, removal of the primary tumor may have a dual stimulating effect on metastasis development, both through removing the tumor homeostatic restraint and stimulating growth by physiological wound healing signaling.

Others have demonstrated escape from breast cancer dormancy in animal models mediated by inflammation (138), the key mechanisms suggested to be neutrophil extracellular traps (139), tumor associated macrophages (124), and neutrophils (138) respectively. The inflammatory stimuli in these studies has come from tobacco smoke (139), LPS injection (138) and surgical trauma (124, 140). In another publication, active inflammation was not necessary to induce escape from dormancy, but rather the fibrotic remodeling of the stroma after inflammation, by collagen-I enrichment (141).

The intrinsic cellular machinery mediating the escape from dormancy has been demonstrated *in vitro* to be a shift in the balance of phosphorylation of ERK in relation to p38 induced by uPAR (142). Other models emphasize the role of EMT/MET in the transition from dormancy (138, 143). An in vivo model of breast cancer dormancy found an activation of the EMT program, mediated by transcription factor Zeb1 in previously dormant cells stimulated by LPS-injection (138). Adding to the complexity, mechanisms of escape from dormancy may be organ specific, in a mouse model of breast cancer, the TGF-β antagonist Coco reactivated dormant cancer cells in the lung, but not in other organs (74).

1.13 Targeting dormancy

With the mechanisms of dormancy maintenance and evasion still incompletely understood, how to best therapeutically target this problem is not determined. Non-dividing, metabolically inactive cells are not considered susceptible to conventional cancer treatment (144). Still, there are a number of trials targeting residual disease in breast cancer with additional systemic therapy after standard of care adjuvant

treatment (NCT00248703, NCT03032406, NCT03400254, NCT01545648). In these studies, residual disease is defined by persistent tumor cells in the bone marrow after chemotherapy, and it may be argued that these cells were resistant to standard therapy rather than truly dormant.

It has been proposed that one feasible approach to target dormant tumor cells would be to stimulate escape from dormancy, as the cells would, once awakened be susceptible to conventional cancer treatment such as chemotherapy and targeted therapy (145, 146). The argument against this is that since no cancer treatment today guarantees complete eradication of all malignant cells, one might risk inducing clinically manifest metastatic disease in a patient that without this attempt to treat never would have suffered from a relapse. Still, it has been attempted in a phase I trial in prostate cancer that was terminated due to low accrual. The strategy in this study was to mobilize dormant prostate cancer cells from the bone marrow to the blood stream by an anti-CXCR4 agent and then to target these cells with docetaxel (NCT02478125).

Another suggestion is to develop therapeutics capable of maintaining dormancy. One phase II trial investigating the capacity of 5-AZA and ATRA to induce and maintain dormancy in prostate cancer treated patients with a biochemical relapse is currently recruiting patients (NCT03572387). This seems attractive, as dormant cells cause no problem to the host. However, experience from long term adjuvant treatment, such as endocrine treatment in breast cancer informs us that adherence to long term preventive treatment is low (147). It is difficult to motivate patients' adherence to therapy over time, where they may experience side effects, but no immediate benefit, and where for a majority, the treatment makes no difference as they would never have had a relapse even without therapy. Perhaps the most feasible approach today, with our limited understanding of tumor dormancy mechanisms, would be short-term prevention of escape from dormancy at times when risk is augmented, such as perioperatively.

2. Purpose and aims

General aim:

The general aim of the project was to evaluate the influence of tissue trauma and wound healing on metastatic relapse of breast cancer.

Specific aims:

- To explore the relapse patterns in breast cancer patients presenting with first relapse for signs of synchronization of growth of metastases (paper I).
- To evaluate relapse dynamics after delayed reconstruction in breast cancer treated patients (paper II)
- To investigate the effect on recurrence dynamics after second surgery in breast cancer treated patients in different clinical and surgical situations (paper III).
- To further examine the impact of events like complications, comorbidity and reoperations in the perioperative period for stimulating effects on relapse dynamics (paper IV)

3. Materials and methods

3.1 Patients

Paper I is based on a retrospective series of 209 consecutive patients presenting with first relapse in breast cancer at Haukeland University Hospital between January 2005 and December 2009. All diagnoses were verified and validated in the patients' records. Time to recurrence was determined by time from primary surgery to occurrence of the first recorded distant metastasis. Exclusion criteria were: synchronous primaries and metastases, evidence of metastatic disease within 2 months after primary surgery, primary tumor not removed, local recurrences and patients with secondary, non-breast cancer. In addition, 12 patients had missing essential information. Thus, 180 patients remained for analysis of metastatic pattern, (Fig 3). Generally, a CT scan at time of first recorded metastasis was used to determine disease burden, but other radiologic modalities were also used. A single investigator (HD) measured all numbers and sizes of metastases according to RECIST 1.1 modified by including both lytic and blastic bone metastases.

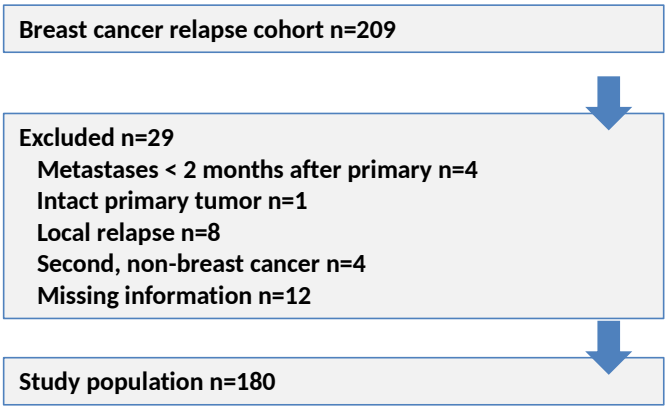

Fig. 3 Inclusion and exclusion criteria paper I

Paper II and **IV** are based on all mastectomy breast cancer patients who underwent delayed reconstructive surgery at Haukeland University Hospital, Bergen, Norway, after primary treatment for breast cancer between 1977 and 2007. After exclusions by

criteria outlined in fig 4, the study group consisted of 312 patients. For paper IV, each patient's record was studied to determine comorbidity, BMI, perioperative medication, reoperations and type and grade of complications according to CTCAE (Common Terminology Criteria for Adverse Events) version 4.0. Complications included in the analysis were: bleeding, systemic infection, local wound infection, mastitis, hematoma, seroma, necrosis, pulmonary embolus, pneumonia and flap dehiscence, all within 30 days after surgery. Smoking status was categorized as current, previous or never smoker. Duration of surgery was extracted from the surgery planning software of our hospital (Orbit version 4.0).

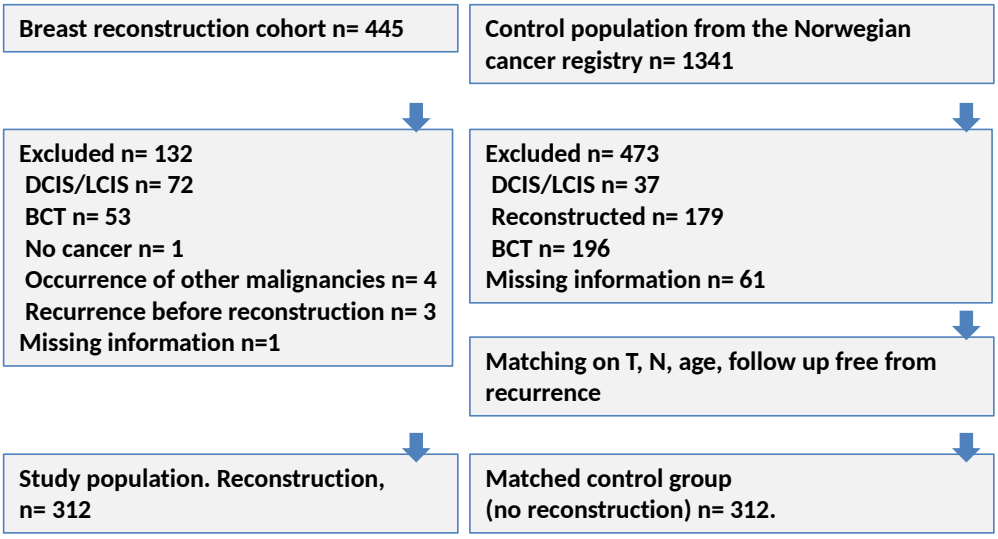

Fig. 4 Inclusion and exclusion criteria reconstruction study population and matched control group.

Paper III is a comparison of the breast reconstruction patients' series (REC) from paper II described above, and patients from three randomized clinical trials suffering from ipsilateral breast tumor recurrence (IBTR) (148) or contralateral breast cancer (CBC) (18, 149, 150) during follow-up. These studies were conducted to evaluate different surgical and radiotherapy approaches at primary tumor removal. Patient and tumor characteristics of these databases are shown in table 3.

Table 3. Patient and tumor characteristics

	IBTR (338)	**CBC (239)**	**Rec (312)**	**Controls (312)**
Median age at diagnosis (years)	45	48	48	49
25%-75%	39-52	42-56	42-53	43-53
Range	21-69	22-75	29-73	28-71
Tumor size (%)				
T1	85	84	61	62
T2	12	15	29	30
T3/4	-	-	8	7
Missing	3	1	2	1
Node negative (%)	70	64	68	67
Node positive (%)	30	36	32	33
ER negative (%)	16	16	20	19
ER positive (%)	63	53	70	70
ER missing (%)	21	31	10	11

3.2 Control group

The control group for paper II is based on a population from the Norwegian Cancer Registry comprising 1341 patients with breast cancer surgery in the same time period as our study population, that had not undergone reconstructive surgery. For data quality purposes, patient's records were studied for validation of diagnosis, patient and tumor characteristics, adjuvant therapy, reconstructive surgery (excluded from the control group), time of first recurrence, and recurrent site in the same way as was done with the cases. Among the 1341 patients, a total of 473 patients were excluded leaving 868 patients, whose characteristics are shown in Table 3, which hereafter will be labeled ''control group.'' From this group, a one-to-one match with identical T- and N-stage, age at diagnosis and a recurrence free follow-up time equal to or greater than time from primary treatment to reconstruction for the matched case, was selected for each reconstructed patient. A reference day was created for each control, representing time from primary surgery to reconstruction for the matched case.

3.3 In vivo model

In an attempt to study the mechanisms of escape from dormancy stimulated by tissue trauma and wound healing, we turned to an *in vivo* model. We decided to use a murine tumor cell line as systemic effects of tissue trauma and wound healing could not be properly evaluated without an intact host immune system. We decided to use the Balb/c syngeneic mammary carcinoma line D2A1-d, described as retaining the ability to extravasate into lung parenchyma after intravenous injection, but failing to proliferate and form macrometastases in the absence of extrinsic stimulation (138). Cells were injected through the tail vein of female 8-week-old BALB/c mice to produce lung micrometastases, animals were subjected to mastectomy of the fourth mammary fat pad. In a second pilot study, we also labelled the cells with GFP, for easier detection of micrometastases and, more importantly, to induce an immune response described by others to result in an extrinsically imposed dormancy (124, 151). To be able to monitor tumor growth in real time, these animals were injected with cells both in the mammary fat pad and into the tail vein. None of these mice underwent surgery. In both pilot

studies, animals were sacrificed after 4-6 weeks and organs and tumors harvested for investigation. Formalin fixed, paraffin embedded lungs and orthotopic tumors were sectioned and stained with H&E and GFP (Invitrogen GFP Polyclonal antibody A-6455, 1:5000).

3.4 Statistical methods

In paper I, standard deviation (SD) from size and number of metastases was calculated for each patient. The choice of SD as a marker of synchronization was based on the assumption that synchronized growth initiation would result in multiple metastases of similar size and thus result in a low SD, while unsynchronized, or random growth initiation would give few metastases of different size, resulting in a high SD. This is again based on the assumption that patients experiencing relapse undergo a period with resting occult micrometastases with restricted growth prior to their first relapse. These dormant micrometastases might be sensitive to systemic growth stimulating signaling, such as growth factors, cytokines etc., and thus, escape from dormancy, and start growing simultaneously.

The median value of SD was used as cut-off value and patients, were grouped as ‘‘low SD’’ and ‘‘high SD’’, accordingly. Associations between different categorical variables were assessed by Pearson’s Chi-square test. Continuous variables not following the normal distribution were compared between two or more groups using the Mann–Whitney U tests. Univariate survival analyses were performed by the product-limit procedure (Kaplan–Meier method). Differences between categories were tested by the log-rank test.

In paper II, event dynamics were studied by estimating with the life-table method the hazard rate for recurrence, i.e., the conditional probability of manifesting recurrence given that the patient is clinically free from any recurrence at the beginning of the time interval. The probability of recurrence over time, i.e., crude cumulative incidence (CCI), was estimated according to a proper nonparametric estimator adjusting for the presence of competing events and compared by the Gray test(152). A discretization of the time axis in six-month units was applied and a Kernel-like smoothing

procedure(153) was adopted. For multivariable regression analysis, the piecewise exponential model was used. The piecewise exponential model provides a flexible semiparametric tool in the study of the hazard function for survival data, in the same fashion as a Cox regression model (154). The log-hazard function was modeled as an additive function of the baseline log-hazard and the covariate effects. Statistical analyses were done using R3.02 software for Windows with Epi package added.

In paper III, distant metastasis free survival times were calculated as time from second surgery (IBTR, CBC or REC respectively) to metastatic relapse or last documented follow-up with no evidence of disease. Relapse dynamics were analyzed with t=0 at the time of second surgery, separate analyses were performed investigating the influence of time from first to second surgery, grouped in 12-month intervals. Second primary tumors, including new contralateral breast cancer, were considered competing events leading to censoring at the time of occurrence. The distant metastasis dynamics was studied by estimating with the life-table method the hazard rate for recurrence, i.e., the conditional probability of manifesting recurrence given that the patient is clinically free from any recurrence at the beginning of the interval.

In paper IV, relapse dynamics were evaluated by using the life-table method for the hazard rate of recurrence, as in paper II. Curve smoothing was done with natural splines, polynomial inverse third order. Differences between groups were analyzed with the Mann-Whitney U-test for continuous variables and X^2-test for categorical variables.

3.5 Ethical considerations

Ethical approval for the studies was granted by the Regional Ethical Committee (REK Vest): 15025. All animal experiments were conducted in accordance with the regulations of the Norwegian state commission for laboratory animals, which are consistent with the European convention for the protection of vertebrate animals used for experimental and other scientific purposes and Council of Europe (ETS 123) and approved by the Norwegian Food Safety Authority, FOTS ID 12083, and the Animal care and use program at the University of Bergen.

3.6 Methodological considerations

The use of standard deviation of metastases as a marker of synchronized growth (Paper I) is novel and thus needs to be confirmed in different data sets. There is also a possibility that size dependency of SD makes direct comparison between very small and very large metastatic lesions inaccurate. The choice of the median SD as a cut off value between synchronized and non-synchronized was chosen due to a lack of previous data or biological rationale for another value, and to avoid strong influence of extreme values.

The fact that breast cancer follow-up in Norway only includes imaging upon symptoms or other reasons to suspect distant metastases such as biochemical alterations, introduces uncertainty regarding duration and growth rate of metastases. This may have diluted the observed effect. Expanding the studies to multiple centers would have augmented sample sizes and made statistical significant results more robust and reliable. It would also, however, introduce a risk of inter-observer variability in measurements of metastatic lesions on radiological images and other variables dependent on some level of observer interpretation. No statistical tests were used to predetermine sample size in any of the studies, as the direction and magnitude of effects were unknown. The reliability of subgroup analyses of different reconstructive methods regarding relapse dynamics is also limited due to small number of patients and few events in this group.

Retrospective studies of patients' groups call for careful consideration of selection criteria. A risk of selection bias in paper II could be an accumulation of early relapses in the no reconstruction control group, as any sign of metastatic disease is a contraindication for delayed breast reconstruction. This was accounted for in the present study by only including controls with a follow up free from relapse at least as long as time from primary surgery to reconstruction for the matched case.

The piecewise exponential model used in paper II was proposed by Iacobelli et al (155), and although not as common as the widely used Cox proportional hazard model, it has

several advantages in the current analyses. It does not assume proportional hazards, that is the idea that the covariates will have a proportional effect that is constant over time, making it better suited to study disease dynamics over time. Other survival models generally assume that an intermediate event, in our case the breast reconstruction, only has a constant, multiplicative effect on the baseline hazard after its occurrence. Such an assumption may lead to a clinically relevant bias as the intermediate event may act not only by multiplying the baseline risk, but also changing the following dynamics. In our work, to allow for non-proportional hazard in estimating the effect of event occurrence, time elapsed from the event occurrence to the end point of interest, namely the new time scale induced, was also accounted for in the model. Thus, we included both the event itself, breast reconstruction, follow-up time from the event and time since primary tumor removal in the multiple timescale model. Time since event occurrence was set to 0, before its occurrence as well as for those patients who did not experience it.

4. Summary of results

Paper I was the first published systematic evaluation of metastatic pattern regarding sizes and numbers in breast cancer patients. Bone- (38 %), lung- (30 %) and liver metastases (23 %) were the most frequent metastatic sites. The median number of lesions measured was 7 (mean 6). We proposed the standard deviation (SD) of size and number of metastases as a marker of synchronized growth. Median SD in the population was 5.4 mm. Patients were grouped as ‘‘non-synchronized’’ if they had a SD above median or if they had only one measurable lesion, and as ‘‘synchronized’’ if they had a SD below median. The mean SD of metastatic lesions seemed to be lower in the first 3 years after primary surgery, although this trend was not statistically significant. As delayed recurrences as well as periods of tumor dormancy seem to be more frequent in lymph node negative patients (156, 157) we analyzed this group separately. We demonstrated a significantly more synchronized relapse pattern in early relapsing, lymph node negative patients compared to late relapsing (median 3.1 vs. 5.7, Mann–Whitney test, $p = 0.018$) and in patients without systemic adjuvant therapy (median 2.5 vs. 6.4, Mann–Whitney test, $p = 0.005$. Fig 5). Also, a significant drop in SD was observed at the time of ended adjuvant endocrine treatment, comparing between year 4-5, just before ended treatment, and year 5-8, the years after (median 13.1 vs. 3.9, Mann–Whitney test, $p = 0.021$). In the analyses of survival, no difference was present for SD of metastases or time between primary diagnosis and recurrence. Low tumor load at time of first recurrence, measured by sum of diameters of metastatic lesions, demonstrated significantly increased overall survival (Log Rank $p=0.001$).

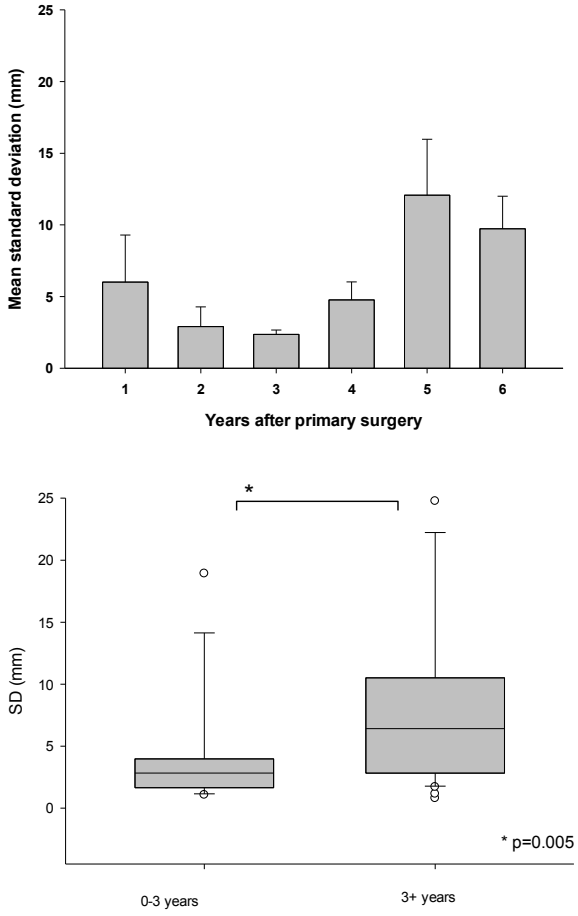

Fig. 5 Metastatic pattern of patients without systemic treatment (N=62). The plots show the mean of standard deviation (SD) of size and number of metastases at first recurrence (±SE) by time after primary surgery. Box plot of mean SD according to early recurrences (0-3 years) versus late recurrences (>3 years).

Paper II investigated the relapse dynamics after delayed breast reconstruction. Median time to reconstruction for the 312 patients was 33 months (range: 1–362 months). Median follow-up after reconstruction was 137 months. Within 10 years after primary surgery, 39 of the 312 reconstructed patients developed local (5), regional (6), or distant (31) relapse, compared to 52 patients in the matched control group (local 10, regional 3, distant 39). When setting time of origin at reconstructive

surgery, we demonstrated a bimodal relapse pattern after delayed breast reconstruction in patients treated for breast cancer, similar to that observed after primary breast cancer surgery, with peaks at 18 months and five years. The early relapse peak was higher in patients undergoing more extensive surgical procedures, suggesting a dose-dependent effect. There was no difference in recurrence free survival between extensive reconstructive surgery and simple implant surgery (Gray test, $p = 0.86$). Timing of the peak was unaffected, both by extent of reconstructive surgery and time interval between primary cancer treatment and reconstruction. Known risk factors for recurrence, nodal status and tumor size, affected the height of the recurrence peak, but again, not the timing. The new timescale introduced by delayed reconstructive surgery demonstrated an increase in hazard ratio for relapse for reconstructed patients, compared to matched control patients the first two years. After this, the risk seems to be lower, although not statistically significant, thus it may not be considered constant (Fig 6). Our results indicate an independent stimulating effect of surgery on pre-existing micrometastases, resulting in a transitory increased risk of relapse the first two years after reconstruction compared with not reconstructed patients, thereafter a decreased risk. Importantly, this did not translate into a worse long term disease-free survival for reconstructed patients compared to the not reconstructed.

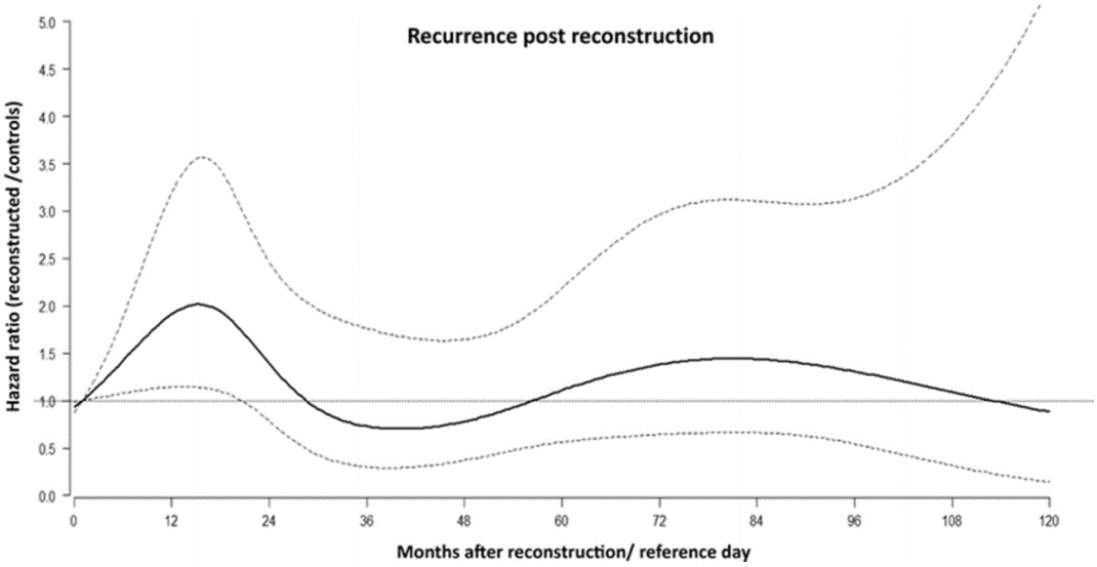

Fig. 6 Hazard rate ratio for recurrence between reconstructed patients and controls in relation to time since reconstruction or reference day. Dotted lines represent 95% CI.

Paper III interrogated the distant metastasis stimulating effect by different second surgical procedures. Our hypothesis was that the distant metastasis stimulating effect would be different if the second surgery involved removal of a tumor in the breast or not. This was based on the assumption that a primary tumor may exert a homeostatic, or growth restricting effect on distant metastases and thus the removal of this tumor would allow growth of these metastases. Surgery per se has also been demonstrated to accelerate metastasis development and our aim was to separate these effects. We compared relapse dynamics in 3 databases of patients undergoing second surgery in different clinical situations, namely surgery for ipsilateral breast tumor recurrence, surgery for contralateral breast cancer and delayed breast reconstructive surgery, where no tumor removal is involved. Despite the fact that these patients were accrued over a wide time-span, the main prognostic factors are remarkably homogenous, with the exception of primary tumor size which was larger in reconstructed patients. In all three patients' series, the relapse dynamics revealed a bimodal pattern with a major peak at 18 months and a lower one at 5-6 years. The levels of recurrence risk were different. Patients operated for IBTR had the highest risk of developing distant

metastasis, the reconstructed patients the lowest and patients operated for a contralateral breast cancer an intermediate risk. Also, the time interval from primary cancer treatment to second surgery was influential on recurrence risk for surgery involving removal of a tumor in the breast, with higher early peaks if the second surgery was performed less than three years after primary treatment. For reconstructed patients, time from primary treatment did not influence risk of relapse (Fig 7).

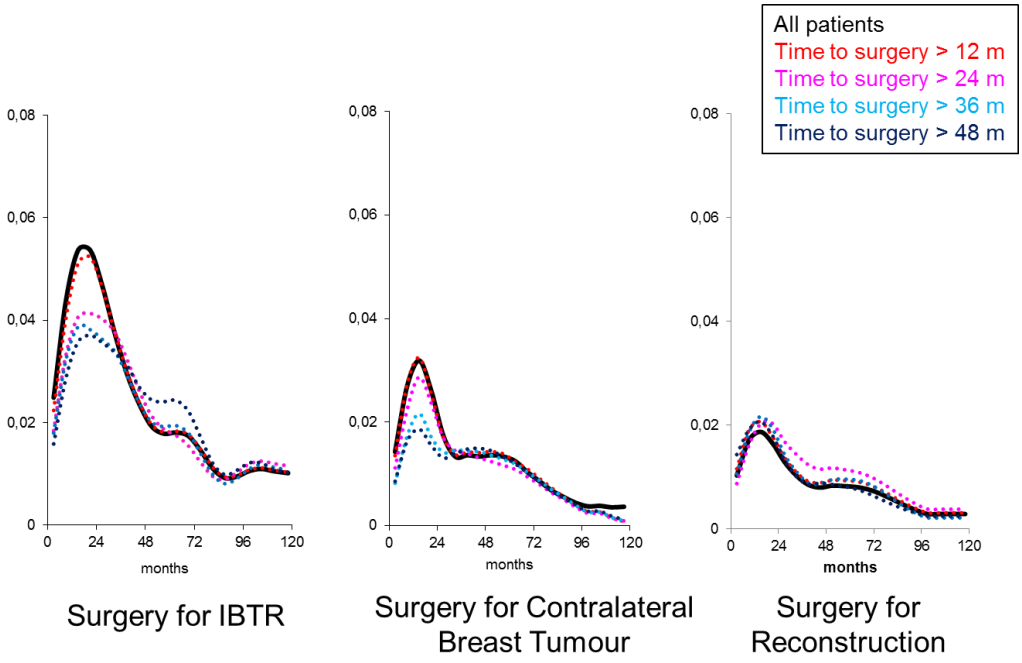

Fig. 7 Hazard rate for recurrence with T=0 at second surgery. All groups display a bimodal pattern with peaks at 18-24 months and 5 years. Time to second surgery changes the height of the first peak when tumor removal is involved, but not in reconstruction.

Paper IV further explored the oncological impact of the perioperative period at breast reconstruction. The majority of patients, 68%, had at least one reoperation, the early reoperations were almost exclusively caused by a complication while later reoperations had a higher rate of aesthetic indications. 28% of all patients

experienced a complication grade ≥2. In the group undergoing autologous flap reconstructions 56% had at least one complication grade ≥2. These patients also had a significantly higher rate of reoperations. When exploring relapse dynamics, we demonstrated an augmented stimulating effect on relapses in patients experiencing complications in the perioperative period as well as in overweight and obese patients.

The **in vivo models** have so far failed to produce publishable results. In the first pilot, a single mouse displayed lung macrometastases at sacrifice, one of the no surgery controls. Micrometastases were not detected in any mice. This led us to doubt the behavior of the D2A1-d-cells in our hands, both regarding their ability to extravasate and colonize the lung tissue and their dormancy in the absence of stimulation. For the second pilot, we took the following measures to address these concerns. First, numbers of mice were increased to improve robustness and reliability of the results. Second, all cells were labelled with GFP for improved detection of micrometastases, and also because GFP has been demonstrated to induce an immune response in BALB/c mice (151) potentially resulting in an extrinsically imposed dormancy of labelled cancer cells (124). At sacrifice after 6 weeks, 15 of the 19 mice had macroscopic orthotopic tumors. The orthotopic tumors were heterogeneous in GFP positivity; some were completely negative, while others displayed 20-70% positive cells. Lung macrometastases was found in one mouse, this did not stain positive for GFP. We found GFP positive lung micrometastases in four additional mice.

5. Discussion

5.1 Discussion of results

In order to evaluate the importance of tissue trauma and wound healing on metastatic relapse of breast cancer we studied patterns and dynamics of breast cancer recurrence in relation to patient and tumor characteristics as well as interventions. A theory building on a period of tumor dormancy before, during or after primary surgery would result in a higher likelihood of finding a solitary metastasis at first recurrence than of finding multiple metastases. However, this holds true only by assuming there is no synchronized internal clock in the dormant tumor cells and no external signal to synchronize growth (158).

For the entire patient series in paper I, there was no significant difference in SD of metastatic lesions between early and late relapsing patients, thus it may be considered a negative study. As this was a very heterogeneous group regarding patient and tumor characteristics, as well as treatment in the primary setting, this should perhaps not be surprising. The finding of a significantly lower SD in early relapsing patients with no systemic adjuvant treatment suggests that primary cancer surgery may indeed synchronize growth of metastases, and that this effect is counteracted by systemic treatment. According to treatment guidelines, fewer of the lymph node negative patients would be expected to have received systemic adjuvant treatment, this was not adjusted for in the analyses in paper I, so the observed difference for this subgroup may in part be ascribed to this fact. Furthermore, the distinct drop in SD of metastases at the time of ended endocrine treatment both supports the idea of systemic treatment acting as a break on dormant micrometastases and serves as proof of principle of SD as a marker for synchronized growth. In other words, synchronized growth may be a result of either the appearance of a growth stimulating signal like surgery, or the removal of a growth inhibitory restraint like systemic adjuvant treatment, both assuming the existence of dormant micrometastases.

Our work demonstrated a relapse pattern fitting with the idea of synchronized growth of dormant metastases in early relapsing, lymph node negative breast cancer patients. As a similar recording of size and number of metastases at first relapse has not been published, our findings need to be confirmed in other, preferably larger, datasets. This may allow for closer analyses of how different subgroups of breast cancer relapse, relate distribution of metastases to time and treatment and reveal novel aspects of metastasis biology.

To evaluate the possible stimulating effect on breast cancer recurrence by surgical trauma and wound healing, we turned to delayed breast reconstruction. This allowed us to study this effect in a pure and standardized setting, unaffected by primary tumor properties and adjuvant treatment, and with a higher likelihood that any micrometastases would truly be dormant as reconstruction is only offered in the absence of suspected metastatic disease. This would also circumvent the argument that relapses after primary surgery is a result of physical dissemination of tumor cells from the breast by the surgical intervention (159). We discovered a relapse pattern after delayed breast reconstruction coherent with a stimulating effect of the surgical procedure on pre-existing micrometastases. The effect size was dependent on the underlying relapse risk of the patient, with higher early peaks in lymph node positive patients and with larger primary tumor size. Timing and magnitude were not affected, supporting the hypothesis that surgical factors act on the subclinic metastatic state of the host. The fact that the hazard rate of *later* relapses was decreased in reconstructed patients further supports the hypothesis that the surgical procedure accelerates growth of occult distant micrometastases that would otherwise have appeared at a later time. A study by Isern et al found a higher risk of relapse in reconstructed patients compared to mastectomy alone, this remained statistically significant in multivariable analysis (38). That study, however, suffered from a suboptimal matching of cases and controls regarding important prognostic factors such as lymph node status. In our study, the pattern of a transitory increase in relapses the first two years after reconstruction, followed by a reduction was evident also in the multiple timescale multistate model. This model takes into account the joint effect of prognostic factors

as well as the effect of the change in hazard rate by specific stimulating events, here reconstruction, occurring at different time points.

In accordance with other reports, we found a slightly better long-term outcome in reconstructed patients compared to controls (37, 160, 161). The reason for this is at present undetermined, but may be explained by higher socioeconomic status and lower comorbidity in patients opting for breast reconstructions, factors that are also associated with a lower risk of relapse (162). In other aspects, other groups have reported contradictory results to ours, with no sign of an acceleration of relapses after delayed reconstruction (37). This discrepancy may be caused by a different selection of patients for reconstructive procedures in different institutions, or different rates of complications to surgery. Indeed, in the study by Geers et al (37), prognostic factors were significantly different, in favor of reconstructed patients, regarding age, tumor size, and lymph node status compared to controls. They also included implant-based reconstructions in the non-reconstruction control group, this was also permitted in a similar study with no evident difference in relapses after autologous reconstruction (163). The argument was that the surgical trauma is significantly smaller in implant based reconstructions compared to reconstructions with autologous tissue flaps. While this is undoubtedly true, it may still be above the threshold for a systemic effect on dormant micrometastases, potentially obscuring differences between these groups. In a large-scale in vivo study, the implantation of a small sponge, and even a mere two centimeter cutaneous incision, immediately sutured, was enough to promote outgrowth of tumors of mammary gland origin at a distant site in mice (124).

Results from preclinical studies, mainly performed in mice models of breast cancer, have provided convincing evidence of a link between inflammation, tissue trauma or wound healing and escape from tumor dormancy (111, 124, 138, 139). These include both transgenic and syngeneic allograft mouse tumor models. While mouse studies have certainly yielded enormous insight into many aspects of tumor biology, species differences as well as the representativeness of murine tumors to their human

counterparts must be taken into consideration, and results cannot always be assumed transferable.

In an attempt to model surgery induced escape from dormancy *in vivo* in this work, we encountered several problems illustrative of the difficulties in modelling dormancy. First, in expanding cells in vitro, there is an inherent risk of selecting for less dormant and more aggressive clones in each cycle. Even though passaging was kept to a minimum, a certain number is inevitable for transduction, expansion, sorting etc. The fact that some tumors, both orthotopic and in lungs, had no GFP expression while others were composed mainly of GFP positive cells can be due to instability of the transduction, or selective advantage of GFP-negative cells in some mice. In vitro, the transduction seemed stable, with >95% of cells being positive after two passages. The conditions encountered in the complex biologic system of the mice, with fully functional immune system, may of course have altered this. Intravenous injection of cancer cells to produce lung metastases includes only the later steps of the metastatic cascade, assuming that the earlier steps, up until extravasation, have already taken place. Surgical trauma in the model did not involve removal of a tumor in the breast. This may be considered an advantage as it eliminates variation due to tumor size, immune infiltration and vascularization of the primary tumor and thus gives a more standardized trauma and wound healing situation. On the other hand, such a model does not take into account the possible effect on metastases exerted by the primary tumor. Further development of an *in vivo* model is ongoing in our group.

To explore the effect on oncologic outcome of different events and conditions at reconstructive surgery, we investigated surgical and medical complications, patient factors like comorbidity and BMI at reconstruction in our cohort, presented in paper IV. We discovered a surprisingly high rate of reoperations and complications in all patients, and particularly patients undergoing autologous flap reconstructions. We showed that complications in the perioperative period, as well as overweight and obesity, add to the metastasis relapse accelerating effect, further establishing the perioperative period as important to metastasis biology.

In paper III we demonstrated relapse dynamics following subsequent surgeries after breast cancer treatment to be similar to the dynamics previously reported after primary tumor surgery. Again, we saw that the surgical procedures exerted their effects on the underlying risk of relapse of the patients, IBTR (ipsilateral breast tumor recurrence) being a well-known risk factor for developing distant metastases; coherently, these patients had the highest hazard rate for relapses. The fact that the patients' series in paper III differ in the primary treatment needs some careful consideration. On the one hand, it may be considered a limitation as all patients in the reconstruction series were treated with mastectomy while patients experiencing an IBTR had breast conserving surgery in the primary setting and CBC (contralateral breast cancer) patients where a mix of the two. Thus, the varying effects observed in relapse dynamics after *second* surgery may be attributed to events in the *primary* treatment. On the other hand, the fact that we still observe such similar relapse dynamics after second surgical procedures, despite different primary treatment can be taken as support of the conclusion that this is actually an independent stimulating event. The difference in how time between first and second surgery affects the risk level for patients undergoing surgical excision of a tumor in the breast but not in reconstructive surgery, suggests two separate effects on distant dormant metastases: one of tissue trauma and wound healing per se and another of the removal of a growth restraining tumor.

It has been suggested that the early peak in relapses may be a statistical artefact, caused by more frequent follow-up in the time after surgery and thus an accumulation of relapses in this time period, while later relapses, and relapses in no surgery controls, may go undetected for a varying length of time, resulting in a more even distribution. While this might be true for locoregional relapses, follow-up does not include imaging or biochemical analyses directed at detecting distant metastases. Indeed, it has been demonstrated that even when adopting more extensive follow-up regimens, 85% of distant metastases are diagnosed after symptomatic presentation (55).

5.2 Strengths

All our data are based on extensive patient and tumor characteristics, for paper I and II extracted from clinical records, by the authors, and are thus both comprehensive and reliable. Biochemical measurements and radiological imaging come from the hospital patients' records and are thus produced by equipment and protocols verified and calibrated to the high standards required for clinical use. The IBTR and CBC groups in paper III come from randomized controlled trials with thorough and systematic recording of clinical data. The long follow-up time, over ten years for all studies on relapse dynamics, is another strength, one necessary to be able to properly evaluate relapse dynamics for patients with such good prognosis, and when aiming at studying tumor dormancy. The relapse pattern of breast cancer, with a bimodal distribution of relapses, as well as the timing of relapse peaks is very similar to publications from other institutions.

5.3 Limitations

The main limitation of our studies is their retrospective nature precluding any conclusions regarding causality. The relatively small number of patients is another major limitation, giving wide confidence intervals, diminishing the external validity of results and the possibility of extensive subgroup analyses. Locoregional relapses may be diagnosed at routine breast cancer follow up, distant metastases, however, are usually detected when they cause symptoms, as follow up in Norway does not include imaging or blood sampling to detect biochemical alterations. Timing of metastatic development is heterogeneous, and patients vary in their tendency to seek medical advice when experiencing symptoms, thus metastases may have been present for a short or long time when diagnosed, this limits the accuracy of our estimations of timing of relapses.

As most analysed patients in our studies were treated before the introduction of HER2-directed therapy, data on HER2-status was unavailable for the vast majority of patients, preventing specific subgroup analyses on the triple-negative cancers.

The highest level of evidence to support or refute a link between physical trauma and relapse of cancer would have to be obtained from randomized controlled trials (164). Such a trial on surgery induced metastatic relapse, however, is hardly feasible, neither regarding ethical approval or patient inclusion. Accumulated retrospective data, from well matched series of cases and controls, supported by pre-clinical investigations, seem to be the best available evidence today.

6. Conclusions

In conclusion, this work demonstrates that tissue trauma and wound healing can have an impact on distant relapse dynamics in breast cancer. In our material, the effect is modulated by extent of surgical trauma as well as the patients' inherent risk as determined by tumor and patient properties. In paper I we proposed SD of metastases at first relapse as a marker of synchronized growth, and demonstrated lower SD of metastases in early recurrent, lymph node negative patients and in patients not receiving adjuvant systemic treatment, coherent with a growth synchronizing effect of primary tumor surgery. In paper II we demonstrated an acceleration of relapses after delayed breast reconstruction resulting in a distinct peak 18 months after reconstruction. This effect was more evident in patients with higher pre-existing risk of relapse, and in patients undergoing more extensive reconstructive procedures. In paper III, we found similar relapse dynamics with peaks at 18 months and 5-6 years after second surgery in breast cancer treated patients, regardless of whether the surgery included removal of a tumor in the breast or not. The time interval between first and second surgery modulated the effect when a breast tumor was removed, leading to a higher first peak in early relapsing patients. The effect on distant metastasis dynamics of reconstruction was unaffected by time interval. This supports the theory of two separate effects on distant metastasis, one by surgery and wound healing per se, and another by disrupting tumor homeostasis. Paper IV supports the perioperative period as biologically important for oncologic outcome by the relationship between complications to reconstructive surgery and further acceleration of relapses. It also showed that host factors such as overweight and obesity modulates this effect, possibly by an inherent low grade systemic inflammation associated with these conditions. In total, this work demonstrates an impact of tissue trauma and wound healing on relapse patterns and dynamics in breast cancer, which may be explained by stimulated escape from dormancy of occult micrometastases, warranting careful selection of patients for surgical procedures and optimization of perioperative care.

7. Future perspectives

The field of tumor dormancy has been intriguing scientists for a long time, but it is only in the last few decades that this phenomenon has been the focus of systematic pre-clinical, clinical and epidemiological studies. We are only scratching the surface of this elusive property of some cancers, including how to detect and manage it. In our view, accumulated evidence, since the pioneering work of Bernhard Fisher up until today, compels us to regard breast cancer as a systemic condition, with the patient potentially harboring multiple micrometastases, even before the detection of the primary tumor. These minuscule cancer deposits are, as all cells and tissue, susceptible to events and signals in the body. Surgery is, and has for centuries been the main pillar in breast cancer treatment. If, as we and others suggest, surgical tissue trauma can indeed awaken dormant micrometastases, it would be a violation against the first principle of medicine, *primum non nocere*, first do no harm.

With increasing understanding of metastasis biology, adjuvant local and systemic treatment have improved outcome for breast cancer patients substantially. In the late 20th and early 21st century, neoadjuvant preoperative treatment was introduced, and its benefit is seen in a number of patients. The systemic impact of events in the perioperative period, however, is only beginning to be explored, and may be an area with potential for substantial improvement in patient outcome. Our group has studies in the pipeline of interventions around the time of surgical procedures in cancer patients to prevent the possible detrimental, unintentional effects of tissue trauma and wound healing.

We and others have presented convincing results of an impact on distant metastasis development by surgery, fitting with an induction of escape from tumor dormancy. The detection of a tiny metabolically inactive, non-dividing cell or group of cells in the human body is inherently difficult. Catching it at the time it transits from this dormant state to proliferation, in order to study the nature of this escape is perhaps even more challenging. Fortunately, we, and scientists across the world, are willing to

face this challenge. Results by our group and others are not yet mature to change recommendations for clinical practice, regarding breast reconstruction or other surgical procedures in patients potentially harboring occult, dormant micrometastases. As this link is being further characterized and understood, we anticipate discovery of targets for intervention, and thus development of preventive medications to maintain the disseminated tumor cells in a dormant state throughout the patients' lifespan, or even to eradicate the dormant cancer cells to ensure freedom from metastatic relapse.

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EPIDEMIOLOGY

Differences in metastatic patterns in relation to time between primary surgery and first relapse from breast cancer suggest synchronized growth of dormant micrometastases

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Abstract A significant variation in the metastatic pattern among breast cancer patients exists. Clinical observations suggest that these differences are related to time to recurrence (TTR), thus suggesting a common systemic growth signal at the time of surgery. Our goal was to identify a marker for synchronized growth of micrometastases. To quantify the metastatic pattern at first relapse, 180 patients with metastatic breast cancer were studied. Standard deviation (SD) of lesions size and lesion number was calculated and served as a marker for variation. Patients with low SD (multiple/similar sized lesions) were assumed to have synchronized growth, whereas patients with high SD were assumed to have unsynchronized growth. Patients were grouped according to TTR; early (< 3 years-) or late (> 3 years- after surgery). In patients not receiving systemic adjuvant treatment, median SD was significantly lower in the early group (2.5 mm) compared with 6.4 mm in the late group ($p = 0.005$). In node negative patients, median SD was significantly lower in the early group (3.0 mm) when compared with the late group (5.7 mm, $p = 0.02$). An additional drop in SD was observed immediately after end of adjuvant endocrine therapy. Our results identify SD as a marker of synchronized metastatic growth in breast cancer. A metastatic phenotype characterized by multiple similar sized metastases, suggesting synchronized onset of growth of micrometastases was predominantly found in patients recurring early after surgery and was counteracted by adjuvant treatment. Systemic growth signals caused by surgery might be antagonized during the time window following surgery.

Keywords Breast cancer · Surgery · First relapse · Metastases · Radiology · Adjuvant treatment · Metastatic pattern · Tumor dormancy

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Introduction

Breast cancer has a long natural history and is infamous for its propensity for late relapses when compared with most other cancer types [1]. Even clinically undetectable, tiny tumors can shed malignant cells into the circulation. Several biomarkers like ER, Her2, TNM-classification, and gene expression signatures [2–5] can readily be applied to predict early local or early distant disease recurrence within 5 years of diagnosis. On the other hand, no biomarkers have been proven clinically useful to predict late relapse [6, 7]. In cases with delayed relapse, the nonlinearity of disease progression gives an indication of the presence of periods with tumor dormancy [8, 9]. Early micrometastatic foci, single cells, clusters of cells, or microscopic tumors can be restricted in growth over periods of time by inability to recruit blood vessels [10], by immunesurveillance [11], by cell cycle arrest [12], by tumor microenvironment (TME) interactions [13] as well as by iatrogenic depletion

of growth stimulatory hormones in the case of ER+ breast cancer [14]. Interestingly, there are several experimental tumor models suggesting that dormant cancer can turn into rapidly progressing disease by growth systemic signals [8, 15, 16]. Moreover, systemic growth signals caused by tissue trauma and wound healing has been shown to initiate and synchronize growth of dormant micrometastases [17, 18]. Also clinical consequences of tissue trauma and wound healing have been discussed, as recently reviewed by Ceelen et al. [19]. Although controversial, in a series of clinical studies on human breast cancer, Demicheli et al. [20–22] suggest that the tissue trauma caused by the primary surgery alone is able to alter the growth kinetics of dormant micrometastases and reduce time to recurrence.

We hypothesize that activation of systemic growth signal cascade in breast cancer patients with dormant micrometastases might result in synchronized growth and thus the detection of multiple similar sized macrometastases at the time of first recurrence. Consequently, the detection of multiple similar sized metastases might serve as a marker of synchronized growth kinetics in these patients. In contrast, detection of solitary metastases or oligometastases with large size variation is more likely to occur when the metastases grow independently in the absence of a synchronizing signal. In the present study, we aimed to quantify size and number of metastatic lesions in relation to time between primary surgery and first relapse. We further hypothesized that growth of dormant micrometastases can be preceded by a synchronizing event like increased levels of wound healing associated growth factors following surgery or sudden withdrawal of anti-endocrine therapy. Moreover, we suggested that metastatic synchronization can be quantified by the standard deviation of size and number of metastases at time of first recurrence, as a marker of variation in the metastatic pattern. We focused on two clinically relevant candidate events that could lead to systemic synchronization of dormant micrometastases common to a majority of breast cancer patients; wound healing after primary surgery and cessation of endocrine adjuvant therapy.

Methods

The study base for this retrospective analysis consists of 209 consecutive patients treated for metastatic breast cancer between January 2005 and December 2009 at the Department of Oncology, Haukeland University Hospital, Norway. The hospital covers a population of 500,000, and all new diagnosed metastatic breast cancer patients in the population are referred to the regional center. All patients registered with an ICD-10 code for breast cancer (C 50.X) as well as one or more codes for metastases (C 77.X–C 79.X) were identified and all diagnoses were verified and validated in the patient records. Time to recurrence (TTR) was recorded as time between primary surgery and time of occurrence of first recorded metastasis. Patients with synchronous metastases and primaries, patients with evidence of metastatic disease within 2 months of surgery, patients that did not have their primary tumor removed, local recurrences, and patients with secondary (non-breast) cancers were excluded. Cases with measurable metastatic disease according to RECIST 1.1, modified by inclusion of both lytic and blastic bone lesions, were studied. Blastic bone metastases occur frequently in breast cancer and were regarded as evaluable for the purposes of this study. Thus, 180 patients were available for analyses of metastatic pattern.

At the time of first relapse, all patients underwent thorough staging with radiology, biochemistry, and clinical examination. Most patients were subjected to multiple radiology modalities like CT-scan, bone scan, MRI, ultrasound, and chest X-ray. All radiology and clinical tumor measurements were re-examined and the following variables were recorded; radiology modality, size of each metastatic lesion according to modified RECIST 1.1, number of metastases, and affected organs. Patients with more than 10 metastases were recorded as ">10". For each case, the standard deviation (SD) of the different sizes of the metastases was calculated. SD was used as a marker for variability in the metastatic pattern. Thus, a patient with multiple similar sized metastases at the time of first recurrence would present with a "low SD" (Fig. 1a), whereas a patient with, i.e., one large and two small metastases would have a "high SD" (Fig. 1b+c). Patients with solitary metastases ($n = 41$, 23 %) were excluded from analyses of SD. No patients underwent metastasectomy. To justify for the effect of tumor size on SD, we also examined the potential use of alternative metric measures of the metastases (SD divided by sum of diameters, SD divided by mean diameter, SD divided by the square root of the mean as well as SD divided by log mean) for their potential use as markers for synchronized growth.

The median value of SD was used as cut-off value and patients, were grouped as "low SD" and "high SD", accordingly. Associations between different categorical variables were assessed by Pearson's Chi-square test. Continuous variables not following the normal distribution were compared between two or more groups using the Mann–Whitney U tests. Univariate survival analyses were performed by the product-limit procedure (Kaplan–Meier method). Differences between categories were tested by the log-rank test.

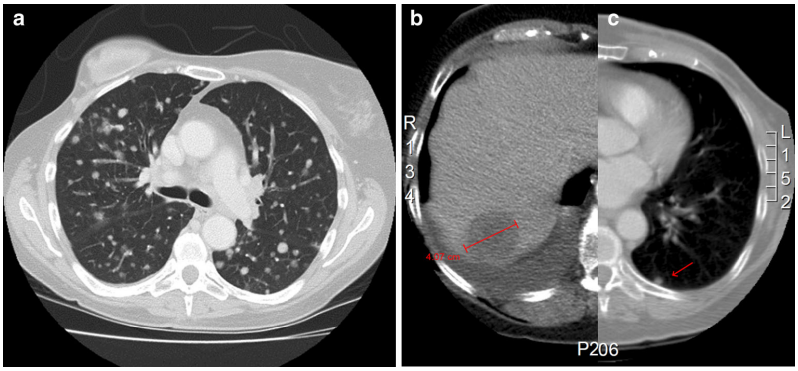

Fig. 1 Patient with multiple similar sized metastases at the time of first recurrence and with low standard deviation (SD) of size and number of metastatic lesions (**a**). Patient with one large liver metastasis (*red line*) and two small metastases lung metastases at the time of the first recurrence (only one is shown here, *red arrow*) and with high SD (**b**, **c**)

Results

The key demographics and pathologic and clinical characteristics of 180 patients recorded at the time of first recurrence are shown in Table 1. A majority of the patients were Stage 1–2 (88 %), ER + (77 %), Her2− (77 %). Bone− (38 %), lung− (30 %) and liver metastases (23 %) were the most frequent metastatic sites. For the majority of the patients (58 %), a CT-scan was the most appropriate modality for tumor size measurements. For patients with bone metastases, MRI (30 %) was the preferred modality, whereas ultrasound (lymph nodes) and x-ray (bone metastases and MRI contraindications) were used in some cases (12 %).

Median time to recurrence was 53 months (2.6–305), and no significant difference was present according to stage, primary tumor grade, or Her2 status in this population. ER negative patients (Log Rank $p < 0.001$) and younger patients (below median) (Log Rank $p = 0.01$) had significantly shorter time to recurrence, median 28 versus 62 months and median 42 and 69, respectively. The annual hazard rate of recurrence for the whole study population is shown in Fig. 2.

Initially, we investigated the number and size of detected metastases in our patient population. The median number of lesions measured was 7 (mean 6). Still, some patients presented with more than 10 lesions and were recorded as ">10", accordingly. Forty-one (23 %) patients had only one measurable lesion at the time of first recurrence. Thus, SD was available in 142 patients. Median SD in the population was 5.4 mm. Patients were grouped as "non-synchronized" if they had a SD above median or if they had only one measurable lesion, and as "synchronized" if they had a SD below median. Median sum of diameters of metastatic lesions was 76 mm (10–697) (Table 2).

We then analyzed in each patient the SD of metastatic lesions in relation to time to recurrence. As illustrated in Fig. 3a+b, the mean SD of metastatic lesions seemed to be lower in the first 3 years after primary surgery, although this trend was not statistically significant. Moreover, as delayed recurrences as well as periods of tumor dormancy are more evident in the node negative patient [23, 24], we analyzed this group of patients separately. In this subset of patients, there was a significantly lower SD in patients who experienced early disease recurrence (≤ 3 years) when compared to those with delayed recurrence > 3 years (median 3.1 vs. 5.7, Mann–Whitney test, $p = 0.018$) (Fig. 3c+d). Similarly, SD was significantly lower during the first three years after primary surgery in patients not receiving systemic adjuvant treatment (median 2.5 vs. 6.4, Mann–Whitney test, $p = 0.005$) (Fig 3e+f). There was no significant association between SD and time after surgery in patients receiving adjuvant systemic treatment.

Adjuvant endocrine treatment might affect the growth kinetics of dormant micrometastases. Consequently, we asked if a second drop in SD occurred at the time of withdrawal of endocrine treatment (5 years of tamoxifen or aromatase inhibitors). As expected, following the end of endocrine treatment at year 5, there was a second drop in SD (Fig 4a). When comparing the period just before end of endocrine treatment (year 4–5) with the period immediately after end of endocrine treatment (year 5–8) SD was significantly lower in the latter period (median 13.1 vs. 3.9, Mann–Whitney test, $p = 0.021$, Fig 4b).

Low SD was significantly associated with low histological grade in primary tumors (Pearson Chi Square $p = 0.002$), the absence of liver metastases (Pearson Chi

Table 1 Patient characteristics

	Number of patients	Percent
Age		
<40	6	3
40–49	25	14
50–59	43	24
60–69	48	27
≥70	58	32
Nodal status		
pN0	89	50
pN1	79	44
pN2	8	4
pN3	4	2
Tumor size		
T1 (<2 cm)	74	41
T2 (2–5 cm)	92	51
T3 (>5 cm)	12	7
T4	2	1
Stage		
1	47	26
2A	63	35
2B	49	27
3A	15	9
3B	2	1
3C	4	2
Grade		
1	27	18
2	74	49
3	50	33
Missing	29	
HR status		
Neg	41	23
Pos	139	77
Her2 status		
Neg	80	80
Pos	20	20
Missing	80	
Adjuvant treatment		
None	62	35
Endocrine	97	55
Chemotherapy	62	35
Radiology modality[a]		
CT-scan	104	58
MRI	55	30
Other[b]	21	12
Metastatic site		
Bone	68	38
Lymph nodes	31	17
Lung	54	30
Liver	41	23
Brain	9	5
Other	21	12

Key demographic and pathological characteristics including age, nodal status, tumor size, stage, grade, HR status, and Her2 status at the time of primary surgery in 180 patients recorded with metastases from breast cancer during 2005–2009 at Haukeland University Hospital, Norway

[a] Refers to the radiology modality used for the analysis of metastases number and size

[b] Ultrasound, chest x-ray, clinical measurement (caliper)

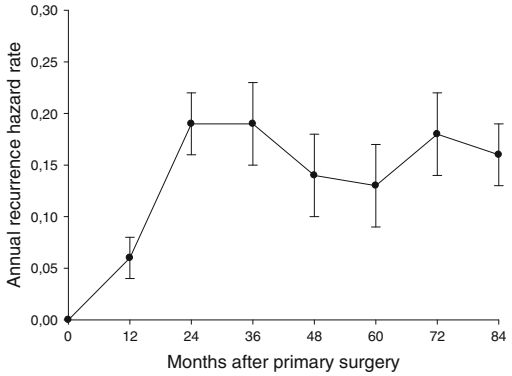

Fig. 2 Annual recurrence hazard rate (± SE) in 180 patients recorded with metastatic breast cancer at Haukeland university Hospital during 2005–2009

Table 2 Metastatic pattern

	Median	Mean	Min–max
Number of lesions counted[a]	7	6	1–>10
∑ diameter of lesions per case (mm)	76	99	10–697
Standard deviation of lesions per case (mm)	5.4	8.3	0–58
Time to recurrence (months)	53	69	2.6–305

Analysis of metastatic pattern at first recurrence in 180 cases of metastatic breast cancer treated at Haukeland University Hospital, Norway. Tumor measurements are in accordance with RECIST 1.1

[a] If >10 lesions, $n = 10$

Square $p = 0.001$), and the presence of lung metastases (Pearson Chi Square $p = 0.02$). No statistically significant association was found between SD and ER status, Her2 status, radiology modality, nodal status, or stage. Other metric measures of the metastases (see methods) did not give significant information in addition to the analyses of SD.

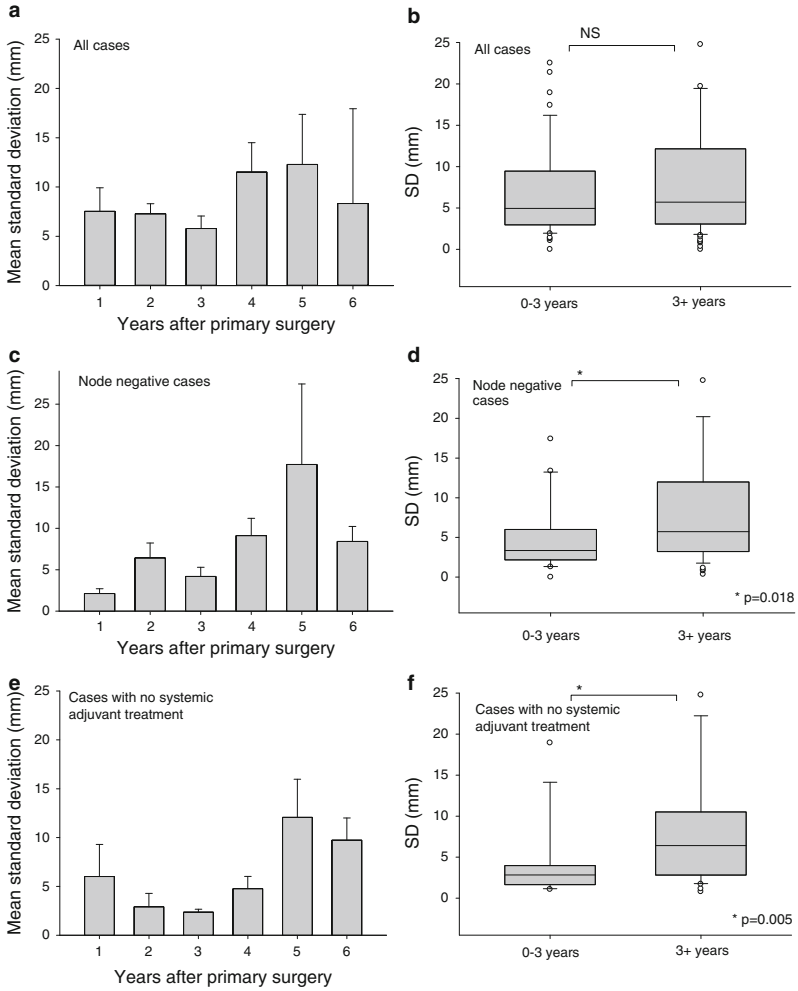

Fig. 3 Metastatic pattern in 180 breast cancer patients. The plots show the mean of the standard deviation (SD) of size and number of metastases in each patient at first recurrence (± SE) according to time after surgery. **a** All cases. **b** Box plot of mean SD according to early recurrences (0–3 years) versus late recurrences (3 + years), all cases. **c**, **d** Node negative cases **e**, **f** Cases with no systemic adjuvant treatment. *Mann–Whitney test

Finally, in the analyses of overall survival between time of first recurrence and death, significantly increased survival was present in ER + cases (Log Rank $p = 0.05$), Her2 positive cases (Log Rank $p = 0.008$) as well as in cases with low tumor load as measured by sum of diameters of metastatic lesions at time of first recurrence (Log Rank $p = 0.001$). No survival differences were present for SD, liver metastases, lung metastases, stage at primary diagnosis, or time between primary diagnosis and recurrence.

Discussion

This study was initiated following the clinical observation of variation in metastatic patterns in patients referred to our ward at the time of first metastatic recurrence from breast cancer. Whereas some patients presented with solitary or oligometastases of varying size, other patients showed multiple similar sized metastases in one or more organs. We further observed that patients in the latter category were frequently diagnosed with metastatic disease shortly

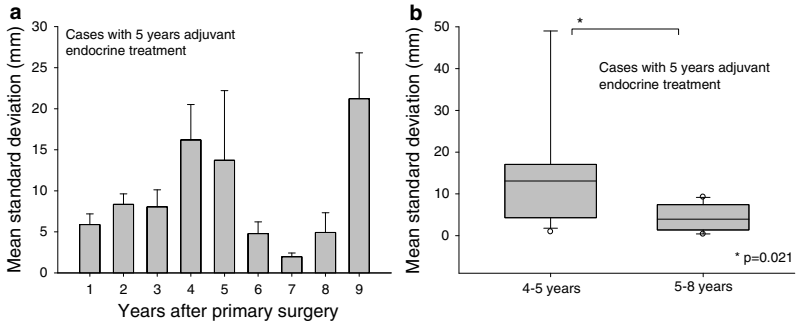

Fig. 4 Metastatic pattern in the subgroup of breast cancer patients treated with adjuvant endocrine therapy. **a** Mean of the standard deviation (SD) of size and number of metastases in each patient at first recurrence (± SE) according to time after surgery. **b** Box plot of mean SD compared between the time before versus after cessation of adjuvant endocrine treatment. *Mann–Whitney test

after primary surgery, shortly after delayed breast reconstruction, or shortly after end of adjuvant endocrine treatment. Delayed recurrence of breast cancer metastases is frequently observed in patients with estrogen receptor (ER) positive disease in particular [5]. An annual recurrence rate of 2 %, for as long as 15 years even after 5 years of tamoxifen [25] or aromatase inhibitors [26], prevents these patients from considering themselves as cured even for decades.

The prominent variation in time between primary surgery and first relapse in breast cancer suggests that there is a great heterogeneity among patients or in the inherent biology of the tumor cells per se. In some cases, a steady growth of metastases and a constant risk of relapse can be inferred by modeling the time of primary tumor detection in relation to the time of relapse as well as the size and number of metastases [27]. In addition, tumor dormancy also in primary tumors is frequently found in the breast, the prostate, and the thyroid gland of undiagnosed patients in various autopsy materials [28], further supporting the existence of growth inhibiting mechanisms or the absence of growth stimulating signals.

In spite of otherwise favorable prognosis when compared with node positive (N+) patients, some node negative (N0) patients do relapse with metastatic tumor growth. In large patient series, the relapses observed in the node negative patients also show a tendency of occurring later when compared with node positive patients [23, 24]. Even tiny tumors might eventually recur at distant sites in spite of radical surgery at the primary stage. The station by station model of breast cancer progression put forward by Halsted over a century ago [29] followed by large and mutilating ultra-radical surgery procedures has been replaced by less invasive methods [30–33]. Recently, also the value of lymph node dissection even in sentinel node positive patients has been challenged [34]. The multimodal approaches including limited surgery with immediate reconstruction, limited irradiation, and effective systemic adjuvant therapy, presently give the best total outcome regarding both survival and quality of life. Nevertheless, in spite of all the recent achievements in the treatment of primary breast cancer, about 10 percent of patients eventually relapse [35]. Even if the concept of tumor dormancy in breast cancer seems to be well established, several controversies concerning the clinical impact exist [36]. Little is known about what mechanisms control dormancy in human micrometastatic disease, and even more important; what physiologic processes can cause the suspension of dormancy and thereby fatal disease relapse. The Gompertzian model of human breast cancer growth as discussed by Norton [37], or more complicated models as suggested by Speer et al. [38], can predict progression of the unperturbed primary tumor and are widely applied in the planning of adjuvant trials. Several mathematical models have been applied to describe different relapse scenarios with regard to time, size, and number of metastases [27, 37, 39]. Still, the lack of knowledge on the mechanisms controlling tumor dormancy and tumor growth spurts renders these models as crude approximations when it comes to predicting relapse in individual patients. The typically highly variable remission periods between resection and relapse in breast cancer patients are inexplicable by continuous growth of metastases [40–42] and imply some degree of growth restriction of occult micrometastases.

In theory, assuming a situation with a period of tumor dormancy before, during or after primary surgery, the likelihood of finding a solitary metastasis at the time of first recurrence is statistically higher than finding multiple metastases. This assumption is valid only if there is no synchronized internal clock in the metastatic tumor cells or no systemic signal to synchronize metastatic growth. Still, in the clinic, we frequently observe patients with multiple

similar sized metastases at first recurrence even many years after removal of the primary tumor. These observations support the concept that there might be a systemic event simultaneously breaking the dormant state of micrometastases. In addition to immunosuppression [11] and hormone deprival [14], it has been suggested that wound healing following the primary surgery might be one such synchronizing signal, by turning on the angiogenic switch in dormant micrometastases and thereby activating angiogenesis [16–18]. The frequently observed peak in the hazard ratio of relapse around 18 months, independent of primary tumor stage [20, 25, 43], has been attributed to the systemic response to primary surgery of breast tumors [44].

In order to find clinical support for a possible association between time of primary surgery and synchronized growth of dormant micrometastases, we studied the metastatic pattern in 180 breast cancer patients at the time of first recurrence. We established the SD of the measured sizes of the metastases as a potential marker for synchronized growth. It is a possible drawback in our material that our patients, in most cases, presented with symptoms or biochemical alterations before the diagnosis of recurrence was established. In Norway, there is no requirement for routine radiological examinations during follow-up. Thus, our data does not give exact information on how long the lesions might have been detectable by radiology ahead of diagnosis, nor of the growth rate. There is a possibility that the power of SD as a marker for synchronized growth is diluted by this weakness. Still, as a marker to identify cases with low variation versus high variation in number and size of metastases (i.e., synchronized vs. non-synchronized), SD was superior to other metric estimates (see methods) by computational simulation, especially when a Gompertzian growth pattern was assumed. Nevertheless, there is a possibility that the size dependency of SD makes direct comparison between the very small and the very large metastatic lesions inaccurate. To our knowledge, this approach has not been previously reported, and thus needs to be confirmed in separate datasets.

We found that the SD was lower in early recurrences (0–3 years after surgery), and this difference was statistically significant in node negative patients. The difference between the node negative and the node positive patients might be due to the difference in the overall prognosis. In node positive patients, the micrometastatic spread is frequently more advanced at the time of primary surgery and the growth into macrometastases might already have been initiated. In contrast, regarding the node negative patients, our results indicate that the dormancy of systemic micrometastases seems to be more susceptible to a systemic synchronizing growth signal. This is in line with the observed delayed recurrences in node negative patients in large patient materials [23, 24]. Sixty-five percent of our patients received systemic adjuvant treatment after primary surgery. The sole intention of this treatment is to prevent or at least delay growth of micrometastases, and this effect was also reflected in a significantly lower SD in early recurrences observed in cases not given adjuvant treatment. This finding might suggest that adjuvant systemic treatment prevents the effect of the synchronizing systemic signal on the tumor cells during the time immediately after surgery. Importantly, delayed initiation of adjuvant chemotherapy has recently been shown to be associated with significantly worse outcome [45], further underlining the importance of the time window immediately following the surgical procedure. We also cannot rule out the possibility that the association between SD and time to recurrence found in node negative cases is, in part, due to the increased use of adjuvant treatment in node positive cases. As expected, there was a second drop in SD directly after end of adjuvant endocrine treatment in ER + patients. From this, we might infer that the removal of the estrogen receptor or aromatase inhibitors acts as a second systemic signal to synchronize growth of occult micrometastases kept dormant during estrogen deprival. This expected finding also serves as an internal control for the utility of SD as a marker of synchronized growth. In comparison to ER−, the ER+ population recurs later [1], and this was also the case in our study. An alternative explanation for the delayed relapse and prolonged dormant state of the slow growing ER+ tumors could be the requirement of an spontaneous enabling sub-clonal evolution in these cells [46], which would occur independently in individual cells over time. The subsequent macrometastases are then likely to be asynchronous. Still, there was no significant association between ER status and SD. This suggests that synchronization occurs at a similar rate in ER positive and negative patients. Low SD correlated with low histologic grade in the primary tumors and suggests that synchronized metastatic growth is more frequent in cases with lower tumor heterogeneity. Still, no significant association between histologic grade and time to recurrence was found. Synchronized growth, quantified by low SD, showed an inverse association in lung metastases and liver metastases. Whereas in the lung, the SD was found to be lower when compared with other sites, SD was significantly higher in the liver, suggesting a different growth dynamic between different organs. Still, most of our patients presented with lesions at multiple sites. In a recent report by Cummings et al. [47] 197 autopsies on patients that died of breast cancer were examined in detail. Of a total of 150 patients, the 46 patients who underwent surgical treatment of the primary tumor were significantly more likely to develop liver metastases, suggesting a role of acute wound healing

after surgery in activating dormant micrometastases in the liver. Similar findings have also been reported by others [48]. Experimental studies have also reported the role of post-surgical wound healing in stimulating growth of liver metastases [16]. Levels of wound healing associated growth factors like Vascular Endothelial Growth Factor show great heterogeneity between patients and also between peripheral blood and locally at the wound site [49]. Studies also show that levels of angiogenesis inhibitors might change following surgery or radiation therapy of the primary tumor [50, 51].

The effect of surgery on macrometastases has been an unresolved issue addressed in multiple retrospective trials studying the impact of removal of the breast in patients with stage IV disease at presentation [52, 53]. Still, several of these trials have been significantly biased based on inclusion criteria. Nevertheless, surgical treatment in patients with synchronous metastases is frequently recommended to increase local control, although overall survival benefit remains to be proven [53]. Recently, a clinical study on 350 women with stage IV disease at presentation, randomized between surgical removal of primary tumor and axillary lymph nodes and systemic therapy, or systemic therapy alone, was presented by Badwe et al. [54]. Although a significant increase in local control was found, the distant site progression free survival was significantly decreased after surgery. Thus, suggesting a detrimental effect of the surgical procedure, as put forward by Fisher et al. [55]. No difference in overall survival was found.

In conclusion, our results identify the standard deviation of number and size of metastases at first recurrence as a marker of synchronized growth of breast cancer metastases. Furthermore, significantly lower SD in early recurrences in node negative patients and patients not given adjuvant systemic treatment suggests a link between the surgical procedure and early synchronized metastatic growth, which might be inhibited by systemic adjuvant treatment. Further research that aim to identify the systemic growth signals caused by surgery and wound healing, might open additional therapeutic opportunities during the time window around or immediately after surgical intervention.

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Conflict of interest The authors have declared no conflict of interests associated with this study.

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EPIDEMIOLOGY

The recurrence pattern following delayed breast reconstruction after mastectomy for breast cancer suggests a systemic effect of surgery on occult dormant micrometastases

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Abstract The purpose of this study was to characterize the recurrence dynamics in breast cancer patients after delayed reconstruction. We hypothesized that surgical reconstruction might stimulate dormant micrometastases and reduce time to recurrence. All mastectomy breast cancer patients with delayed surgical reconstruction at Haukeland University Hospital, between 1977 and 2007, $n = 312$, were studied. Our control group consisted of 1341 breast cancer patients without reconstruction. For each case, all patients in the control group with identical T and N stages and age ± 2 years were considered. A paired control was randomly selected from this group. 10 years after primary surgery, 39 of the cases had relapsed, compared to 52 of the matched controls. The reconstructed group was analyzed for relapse dynamics after mastectomy; the first peak in relapses was similarly timed, but smaller than for the controls, while the second peak was similar in time and size. Second, the relapse pattern was analyzed with reconstruction as the starting point. A peak in recurrences was found after 18 months, and a lower peak at the 5th–6th year. The height of the peak correlated with the extent of surgery and initial T and N stages. Timing of the peak was not affected, neither was the cumulative effect. The relapse pattern, when time origin is placed both at mastectomy and at reconstruction, is bimodal with a peak position at the same time points, at 2 years and at 5–6 years. The timing of the transition from dormant micrometastases into clinically detectable macrometastases might be explained by an enhancing effect of surgery.

Keywords Breast cancer · Surgery · Breast reconstruction · Recurrence dynamics · Tumor dormancy · Multivariate regression

Introduction

The major cause of breast cancer mortality is metastatic disease, and the prevention of metastatic spread and growth is the aim of primary local and systemic therapy [1]. Still, after initial treatment with curative intent, breast cancer is known for its potential to cause late relapse. Even tiny tumors, undetectable by physical, biochemical, or radiological examination, can shed malignant cells into the circulation and eventually cause recurrences up to 20 years after the primary surgery [1]. The current view of breast cancer as a systemic disease at the time of diagnosis was introduced by Fisher in the late 1960s [2]. The concept of tumor dormancy has been proposed [3–6] as an explanation of the latency of metastatic disease, and the past and current research is beginning to unravel the mechanisms of maintenance as well as disruption of dormancy [7]. Early micrometastatic foci can be restricted in growth over periods of time by inability to recruit blood vessels [8], by

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immune surveillance [9, 10], by cell cycle arrest [11], or by tumor–microenvironment (TME) interactions [12].

Signs of stimulation of micrometastases after surgical intervention have been observed in experimental and epidemiological studies and evaluated in mathematical models [13–15]. Furthermore, clinical investigations support an enhancing effect on the growth of metastases after surgical primary tumor removal [16, 17]. Tissue trauma and subsequent wound healing have been shown to cause both local and systemic growth signaling cascades, and might thereby possibly alter the dormant state of occult micrometastases [18, 19]. In a previous study, our findings indicated the presence of synchronized tumor growth in metastatic breast cancer [20]. In some reports on human cancers such as primary breast cancer [21], ovarian [22], colorectal [23], lymphoma [24], and others, tissue trauma has been associated with tumor progression. It was proposed that the primary surgery by itself can represent a stimulating event responsible for the peak in the incidence of metastatic disease observed around 2 years postoperatively independent of tumor stage [25–27]. In light of these findings, questions have been raised regarding the safety of delayed reconstructive surgery. Both increased and reduced risk of recurrence was reported after delayed breast reconstruction [28, 29]. The aim of the present study was to characterize the recurrence dynamics in breast cancer patients that underwent delayed reconstructive surgery. We hypothesized that delayed reconstructive surgery might stimulate preexisting, occult dormant micrometastases and alter the recurrence dynamics.

Patients and methods

Study population

The study population for this retrospective analysis consists of all mastectomy breast cancer patients who underwent delayed reconstructive surgery at Haukeland University Hospital, Bergen, Norway, and had their primary treatment between 1977 and 2007. The respective reconstructive procedures were implant surgery, implants combined with flaps, deep inferior epigastric perforator (DIEP) flaps, and transverse rectus abdominis myocutaneous (TRAM) flaps. Distinction was not made between single- and multistage surgery. Altogether, 312 patients were included after exclusion of patients for whom both tumor size and nodal status were not known as well as patients with secondary, nonbreast cancers and DCIS (ductal carcinoma in situ) (Fig. 1). The hospital covers a population of 600,000, and in this period, all late reconstructive surgical interventions following breast cancer in the region were performed here. Each patient's record was studied to validate diagnosis, patient and tumor characteristics, adjuvant therapy, time and type of reconstructive surgery, time of first recurrence, and recurrent site.

Control group

We received a control population from the Norwegian Cancer Registry comprising 1341 patients with breast cancer surgery in the same time period that had *not* undergone reconstructive surgery. Reporting breast cancer treatment to this registry is mandatory for all physicians in Norway, and the latest published evaluation from 2007 showed a 99 % completeness of data [30]. For data quality purposes, patient's records were studied for validation of diagnosis, patient and tumor characteristics, adjuvant therapy, reconstructive surgery (excluded from the control group), time of first recurrence, and recurrent site in the same way as was done with the cases. Among the 1341 patients, a total of 473 patients were excluded (see Fig. 1 for details) leaving 868 patients, whose characteristics are shown in Table 1, which hereafter will be labeled "control group."

Matching

For each patient in the reconstruction group, all patients in the control group with identical T and N stages, age $\pm$ 2 years, and follow-up without recurrence equal to or longer than the time to reconstruction of the respective matched reconstructed patient were considered. In this initial step, each case could have a number of candidate controls of 0-X. A *reference day* was calculated for each of the controls in these groups representing time from primary surgery for the control plus time from primary surgery until reconstruction for the matched case. Therefore, time from primary surgery until *reconstruction/reference day* could by calculated for cases and controls, respectively. A paired control was randomly selected from this group. If this group was empty, increased age interval up to 5 years was allowed as a first step, and in a few cases when the age difference was considered clinically relevant (e.g., pre- vs postmenopausal), patients with similar, but not identical T classification (e.g., T2 instead of T1) within the right age interval were considered. This group of 312 patients, whose characteristics are shown in Table 1, will be hereafter labeled "matched control group."

Follow-up

Time to recurrence (TTR) was recorded as the time from primary surgery to recurrence. The endpoint of primary interest was the first evidence of recurrence: survival times were calculated as the time elapsed since primary surgery

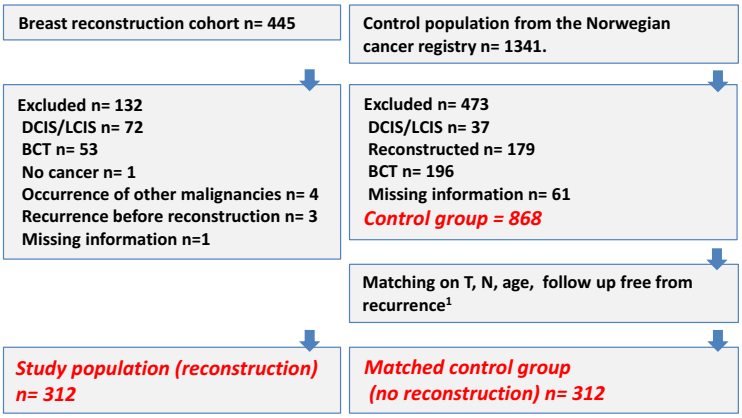

Fig. 1 Inclusion and exclusion criteria employed to achieve case, control, and matched control populations. *DCIS* ductal carcinoma in situ, *LCIS* lobular carcinoma in situ, *BCT* breast-conserving therapy. 1 Recurrence-free follow-up time equal to or longer than the time to reconstruction of the respective matched reconstructed patient

Table 1 Patient, tumor, and treatment characteristics

	Reconstruction group $n = 312$ (%)	Control group $n = 868$ (%)	Matched control $n = 312$ (%)
Year of primary diagnosis			
1977–1989	15 (4.8)	57 (6.6)	25 (8.0)
1990–1999	99 (31.7)	298 (34.3)	109 (34.9)
2000–2009	198 (63.5)	513 (59.1)	178 (57.1)
Median age at diagnosis	48.0	50.0	49.0
Mean age at diagnosis	48.1	50.7	48.7
Age <50	171 (54.8)	397 (45.7)	171 (54.8)
Age ≥50	141 (45.2)	471 (54.3)	141 (45.2)
Tumor size			
T1	190 (60.9)	379 (43.7)	192 (61.5)
T2	91 (29.2)	332 (38.2)	94 (30.1)
T3	22 (7.1)	87 (10.0)	21 (6.7)
T4	2 (0.6)	43 (5.0)	2 (0.6)
Missing	7 (2.2)	27 (3.1)	3 (0.9)
Nodes			
Negative	212 (67.9)	428 (49.3)	210 (67.3)
Positive	100 (32.1)	421 (48.5)	102 (32.7)
Missing		19 (2.2)	
ER status			
Negative	61 (19.6)	190 (21.9)	60 (19.2)
Positive	218 (69.9)	544 (62.7)	216 (69.2)
Missing	33 (10.6)	134 (15.4)	36 (11.5)
Adjuvant endocrine treatment			
No	117 (37.5)	238 (27.4)	115 (36.9)
Yes	136 (43.6)	379 (43.7)	132 (42.3)
Missing	59 (18.9)	251 (28.9)	65 (20.8)
Adjuvant chemotherapy			
No	144 (46.2)	327 (37.7)	136 (43.6)
Yes	143 (45.8)	305 (35.2)	125 (40.1)
Missing	25 (8.0)	235 (27.1)	51 (16.3)

to recurrence or to the last documented follow-up with no evidence of disease. Both locoregional recurrence and distant metastasis were defined as the events of interest, whereas all new primary tumors, including contralateral breast cancers, were considered competing events, thus for these patients survival times were censored at the time of their occurrence. Adjuvant local and/or systemic treatment was given according to national guidelines at the given time period and was not affected by delayed breast reconstruction. Follow-up after curative breast cancer treatment in Norway does not include radiologic evaluation or blood samples other than upon clinical suspicion of distant metastases. Thus, diagnosis of relapse is most commonly made after patients' experience of symptoms. Even when adopting more meticulous follow-up regimens, more than 85 % of recurrences are detected following symptomatic alert and not at controls [31]. Oncological follow-up is not influenced by reconstructive surgery.

Statistical analysis

The event dynamics were studied by estimating with the life-table method the hazard rate for recurrence, i.e., the conditional probability of manifesting recurrence given that the patient is clinically free from any recurrence at the beginning of the interval. The probability of recurrence over time, i.e., crude cumulative incidence (CCI), was estimated according to a proper nonparametric estimator adjusting for the presence of competing events and compared by the Gray test [32]. A discretization of the time axis in six-month units was applied and a Kernel-like smoothing procedure [33] was adopted. For multivariable regression analysis, the piecewise exponential model was used. The piecewise exponential model provides a flexible semiparametric tool in the study of the hazard function for survival data, in the same fashion as a Cox regression model [34]. The log-hazard function was modeled as an additive function of the baseline log-hazard and the covariate effects. For estimation of the piecewise exponential model, the follow-up time was split into 3-month disjoint intervals and the event rate was assumed to be constant within each interval. The model accounts for reconstruction as a time-dependent covariate (i.e., switching from 0 to 1 at the time it was performed). The model was extended to account for the new timescale induced by reconstructive surgery, namely the time elapsed since reconstruction to the endpoint of interest [35]. For practical purposes, time since reconstruction assumed the value 0, before its occurrence, as well as for the controls.

Available prognostic factors were taken into account to adjust the multivariable regression model. These included age at diagnosis, pathologic tumor size (T2–T4 vs. T1), nodal status (N+ vs. N0), and estrogen receptor status (ER+ vs. ER−), with time-dependent effect (by introducing Time (since primary tumor surgery) * ER interaction). To allow for the estimation of baseline hazard, both timescales were modeled via Natural Splines with 5 knots (corresponding to the quantiles of event times only). For age at diagnosis, a possible nonlinear effect was also tested. Statistical analyses were done using R3.02 software for Windows, with Epi package added.

Results

Of the 312 patients, 302 had reconstructive surgery within 180 months and 291 within 120 months after primary surgery, whereas the remaining 10 had longer time to reconstruction. Median time to reconstruction was 33 months (range: 1–362 months). Median follow-up after reconstruction was 137 months. Within 10 years after primary surgery, 39 of the 312 reconstructed patients developed local (5), regional (6), or distant (31) relapse, compared to 52 patients in the matched control group (local 10, regional 3, distant 39).

As a first step, the recurrence dynamics for the reconstructed patients were analyzed with the time origin at primary cancer surgery (Fig. 2, blue line). As expected, a bimodal hazard rate pattern was observed, with an early less prominent peak in comparison with the second later one. When the recurrence dynamics were analyzed with reconstructive surgery as the time origin, a distinct early peak in recurrences was found around 18 months postreconstruction, followed by a second lower peak at the 5th–6th year (Fig. 2, red line). The height of this peak was dependent on the extent of surgery. More extensive surgeries like deep inferior epigastric perforator (DIEP) flaps,

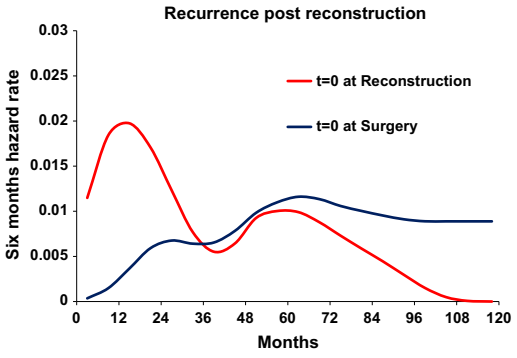

Fig. 2 Recurrence pattern for the reconstructed patients ($n = 312$) with $T = 0$ set at reconstruction (*red line*) and at primary surgery (*blue line*). *X*-axis represents time in months. *Y*-axis represents six-month hazard rate

transverse rectus abdominis myocutaneous (TRAM) flaps, and combined implant and flap surgery as compared with simple implant resulted in a higher peak for the former (Fig. 3). The timing of the peak was not affected by the extent of surgery. There was no difference in recurrence-free survival between extensive reconstructive surgery and simple implant surgery (Fig. 4, Gray test, $p = 0.86$). Similarly, the height of the recurrence peaks, but not the timing, was dependent on known risk factors such as nodal involvement and T stage (Fig. 5).

The relapse pattern for the matched control group, when the time origin was set at mastectomy, followed the expected bimodal pattern with a first, dominant early peak and a second less marked peak at 5 years after primary surgery (Fig. 6), as frequently demonstrated in previous studies. When the time origin was moved to the reference day, the hazard rate curve appears as a simple distortion of the previous one (figure not reported). Unlike the reconstructed patients, no definite trait was detectable. The recurrence incidence was slightly reduced for the reconstructed patients in comparison with matched control patients, although the difference was not statistically significant (Fig. 7, Gray test, $p = 0.08$).

The multiple scale analysis supports the hypothesis of a transitory significant increase of recurrence risk during the first two years after reconstruction/reference day for reconstructed patients in comparison with the *not* reconstructed patients. In the multivariable regression model, where all 868 patients in the control group were analyzed, all factors that were considered confirmed their expected prognostic impact, including the time-dependent effect of ER status, whereas age at diagnosis did not. The hazard ratio (HR) was higher in node-positive patients and in those with increasing tumor size (Table 2). The multiple time-scale model allows for understanding whether the time effect, induced by reconstruction occurrence, may be relevant for the subsequent risk of developing unfavorable events. To better interpret the model, a graph showing the effect of the timescale induced by reconstruction surgery is shown in Fig. 8. The figure shows how the hazard ratio for recurrence between reconstructed patients and control patients (with the same clinical and pathological features and with the same follow-up time since primary tumor surgery) may not be considered constant during the subsequent follow-up time. Although not fully significant with a moderately wide confidence interval, it shows an increased risk for the reconstructed patients within the first 2 years, with a peak at about 18 months after surgery and decreasing thereafter. Of note, the recurrence dynamics following reconstructive surgery were unaffected by the time from primary surgery to reconstruction, both in timing and magnitude.

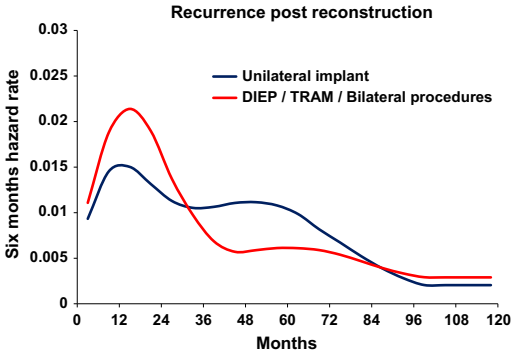

Fig. 3 Recurrence pattern according to surgical intervention demonstrates an enhanced, but similarly timed, effect by increased extent of trauma. *Blue line* patients receiving a unilateral implant. *Red line* patients receiving more extensive surgery. *X*-axis represents time in months since reconstructive surgery. *Y*-axis represents six-month hazard rate. *DIEP* deep inferior epigastric perforator, *TRAM* transverse rectus abdominis myocutaneous flap

Discussion

To our knowledge, this is the first study investigating the dynamics of recurrences occurring after delayed breast reconstruction in breast cancer patients. The main result of our analysis is that when the time origin is set at the reconstruction date, the hazard rate for ensuing recurrence displays a first main peak in the 2nd year and a later minor peak at the 5th–6th year after reconstruction (Fig. 2, red line). The recurrence risk for the same patients, when the time origin is placed at mastectomy, is bimodal with peak positions at the same time points relative to mastectomy, after 2 and 5–6 years (Fig. 2, blue line), as expected [31]. Thus, when the time origin is moved for each reconstructed patient to the reconstruction date, the recurrence risk pattern is similar to that observed following primary mastectomy. Of note, time origin displacement reveals an increase of the early peak with a concomitant decrease of the late level of recurrence risk (Fig. 2), suggesting that recurrence redistribution is associated with the reconstruction maneuver which could be said to act as a wave breaker for recurrences. These findings suggest that mastectomy and reconstruction induce similar biological effects on subclinical preexisting metastases.

The effects of primary mastectomy have been investigated in both animals and humans during the past century [36]. An unintentional effect of surgery in breast cancer patients with clinically undetectable micrometastatic disease has been explained by a paradigm based on the concepts of tumor homeostasis, tumor dormancy, and surgery-

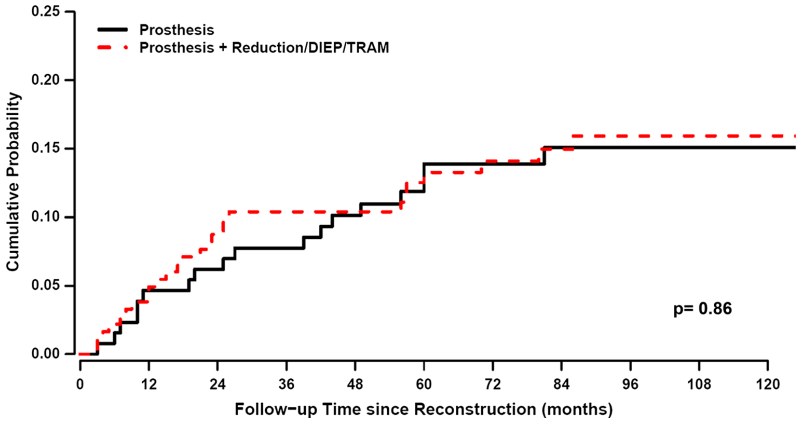

Fig. 4 The probability of recurrence over time, i.e., Crude Cumulative Incidence (CCI), was estimated according to proper nonparametric estimator adjusting for the presence of competing events and was compared between groups by the Gray test. Simple unilateral implant (*black line*) and more extensive surgery such as DIEP/TRAM or bilateral procedures (*red line*). *X*-axis represents time since reconstruction in months. There is no observable difference between the groups

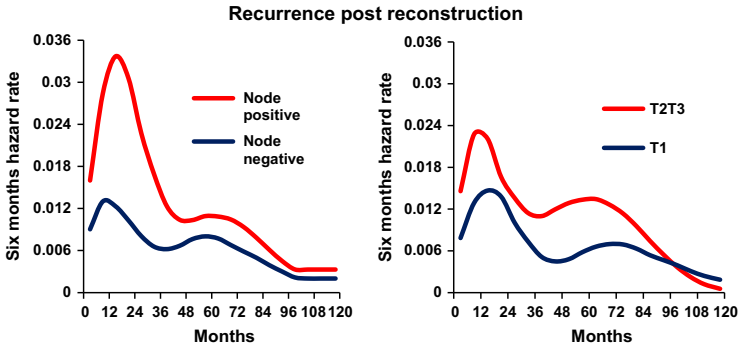

Fig. 5 Subgroup analysis of recurrence pattern by known prognostic factors. Increasing T and N stages are associated with an enhancing effect on preexisting recurrence risk. *Left* figure demonstrates the recurrence dynamics for node-positive (*red line*) and node-negative (*blue line*) reconstructed patients. *Right* figure demonstrates the recurrence dynamics for reconstructed patients with tumors >2 cm (*red line*) and ≤2 cm (*blue line*). *X*-axis represents months since reconstruction. *Y*-axis represents six-month hazard rate

related enhancement of metastasis growth [37]. The model assumes both cellular and micrometastatic tumor dormancy, with ordered transitions between these two quiescent states and subsequent development of overt metastasis and, subsequently, a transient phase of acceleration of metastatic growth. Preclinical studies have pointed to an angiogenic switch as a possible involved mechanism, in which the microenvironment is altered by tissue trauma to become proangiogenic with increased levels of VEGF and reduced levels of angiogenesis inhibitors such as TSP-1 [8]. Others have focused on the role of surgery-induced immunomodulation with demonstration of a stimulatory interaction between cells of the innate immune system and adjacent cancer cells [38]. The truth may lie in both models as the immune and angiogenic systems have multiple points of intersection [39]. Our findings support the concept that also delayed breast reconstruction may accelerate metastatic growth in subjects with dormant metastatic foci similar to the effects observed after primary surgery. This explanation is further supported by the finding that surgical approaches with different extents result in different recurrence risks, although with the same time rhythm (Fig. 3). Specifically, the more extensive reconstruction modalities DIEP/TRAM and bilateral surgical procedures give rise to a higher early peak in comparison with unilateral implant surgery. This difference is limited to the recurrence risk level, which is differently modulated within the same time cadence, while the two types of reconstruction do not affect long-term outcome differently (Fig. 4).

The proposed explanation assumes that the risk of clinical appearance of metastasis is dependent on the action of surgery-related factors on the subclinical metastatic state of the host. This assumption is confirmed by the marked influence of both tumor size and nodal status on the hazard

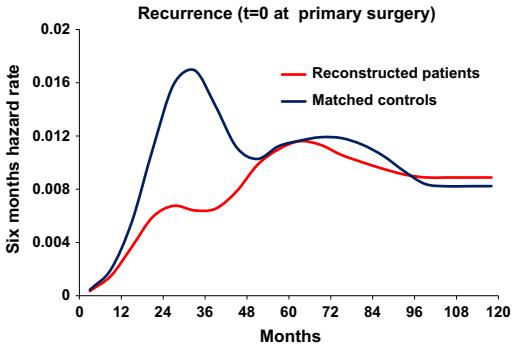

Fig. 6 Recurrence pattern for the reconstructed patients and the matched control patients with $T = 0$ at primary surgery. *Red line* reconstructed patients. *Blue line* control patients. *X*-axis represents months since primary surgery. *Y*-axis represents six-month hazard rate

Table 2 Hazard ratio for recurrence according to tumor characteristics

	HR	95 % CI	
ER pos versus neg (18 months)	0.30	0.19	0.47
ER pos versus neg (60 months)	1.77	0.81	3.86
T2 versus T1	1.91	1.43	2.56
T3 versus T1	2.78	1.89	4.09
T4 versus T1	3.13	1.91	5.11
N+ versus N−	1.98	1.52	2.59

level (Fig. 5). At diagnosis, these two well-known prognostic factors indicate the recurrence risk during the disease course. Thus, it is coherent that an additional triggering factor, such as delayed surgical reconstruction, may result in different outcomes when patients with different underlying recurrence risk are involved.

When studying the relation between outcomes of patients undergoing delayed reconstruction in comparison with patients undergoing mastectomy without reconstruction, crucial problems emerge. In addition to the retrospective nature of such studies, the reconstructed group is characterized by a selection event not yet occurred (and therefore unknown) at the mastectomy time and, moreover, occurring at varying patient-related times during follow-up, thus raising important issues in the statistical analysis. To overcome these drawbacks, we used two different approaches. In the first approach, which has been frequently adopted in this field in spite of its intrinsic naivety [28, 29], we performed a matched random choice of the control patients. For each reconstructed patient, we randomly identified a matched control patient with similar initial characteristics, selecting her among the not reconstructed patients who were disease free at the date of reconstruction of the considered patient. This matching modality resulted in a good balance between the two sets of patients (Table 1) and avoided drawbacks detectable in published reports, such as dissimilar patient characteristics (e.g., age) [40] or dissimilar prognostic factors [28, 29]. In

Fig. 7 The probability of recurrence over time, i.e., Crude Cumulative Incidence (CCI), was estimated according to proper nonparametric estimator adjusting for the presence of competing events and was compared between groups by the Gray test. *Red line* reconstructed patients. *Blue line* matched control patients. Despite matching by age, time of diagnosis, and T and N stage, there is a nonsignificant trend for a more favorable prognosis in the reconstructed patients. *X*-axis represents time in months since reconstruction/reference day (see m&m) for reconstructed patients and control patients, respectively. *Y*-axis represents accumulated recurrence-free survival

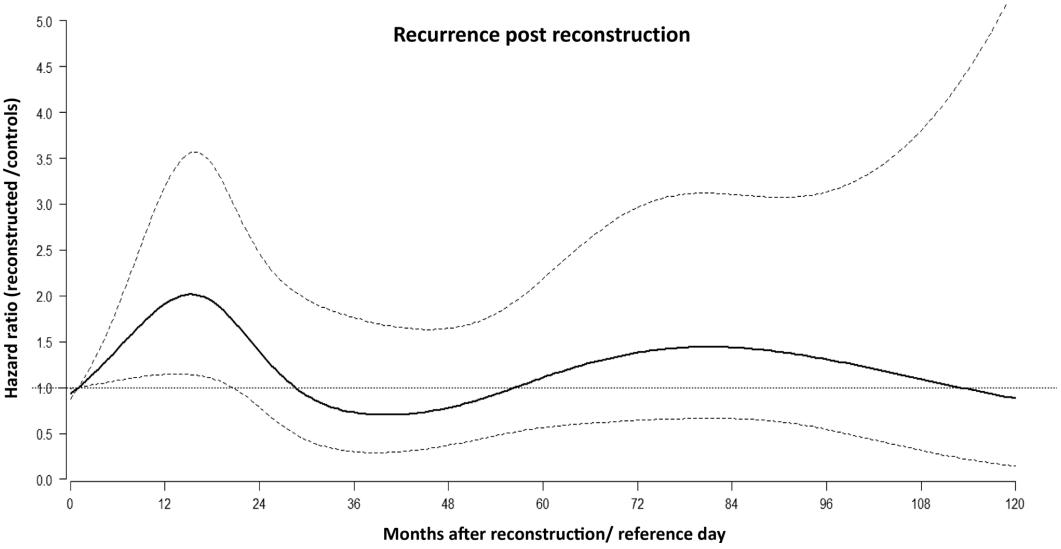

Fig. 8 Multiple timescale analysis of the hazard ratio for recurrence between the reconstructed patients and the controls in relation to time since reconstruction. *Dotted lines* represent 95 % CI. *X*-axis represents months since reconstruction/reference day. *Y*-axis represents the ratio between the six-month hazard for recurrence between reconstructed patients and controls

addition, the recurrence dynamics of matched controls, when time origin was set at mastectomy (Fig. 6, blue line), was coherent with the analogous hazard rate pattern observable in similar patients from different databases [31]. It displays a first peak significantly higher than the corresponding peak of reconstructed patients (Fig. 6, red line) revealing that, in spite of the balanced initial prognostic factors, matched control patients display worse prognosis than reconstructed patients. This finding is consistent with a population-based registry study analyzing Danish women who received reconstruction with implants only [41].

In the second approach, we addressed the analysis in the framework of multiple timescales in multistate models [35]. The regression analysis involved all 868 control patients and 312 reconstructed patients. It accounted for the joint effect of prognostic factors (e.g., tumor size, nodal status, etc.) and, most importantly, the change in the hazard rate for recurrence resulting from specific events, such as reconstruction, occurring at varying time points. The multiple scale analysis provides evidence that following reconstruction women suffer a transitory, significant increase of recurrence risk during the first 2 years in comparison with not reconstructed patients (Fig. 8). This finding provides structural evidence for the enhancing effect of reconstructive surgery on subclinical metastases, which brings on the temporary raise of clinically evident recurrences. Such behavior is suggestive when it is considered in the light of the above-reported model. Indeed, the whole pattern would suggest that the early peak may be caused by an event that has been brought forward, and in the absence of reconstruction were to be expected at a later time. Taken together, the two analysis approaches provide evidence that reconstructed patients (a) suffer increased surgery-related recurrence rate following the usual bimodal pattern and (b) display disease-free survival that is not worse (maybe even better) than that of not reconstructed patients (Fig. 8).

Although not directly comparable, the recurrence pattern analysis apparently diverges somewhat from a previous investigation where trauma or intervening surgical procedures unrelated to cancer were not associated with an increased rate of breast cancer recurrence [42]. Still, the fact that the effect of delayed reconstruction on the recurrence dynamics does not translate into reduced recurrence-free or overall survival is in keeping with most reports on the same subject [28, 29, 40, 41, 43]. The reason underlying the similar or relatively better long-term outcome of reconstructed patients is presently undetermined. Patients opting for reconstruction tend to be younger and have less comorbidity. Most studies to date have focused on immediate reconstruction [43–45]. We are inclined to ascribe this finding to selection bias of patients receiving reconstruction due to factors here unaccounted for, such as socioeconomic conditions and better general health. The former factor is related to the finding that patients who are of lower socioeconomic status are more likely to have a recurrence than women of higher social class [46].

Furthermore, current smokers or diabetic patients are not accepted for microvascular free (DIEP) or pedicle flap surgery (TRAM). In addition, body mass index, which is a prognostic factor in cancer [47, 48], would not be higher than 30 in patients offered advanced reconstruction. Most patients undergoing DIEP or TRAM procedures have a secondary or even tertiary surgery performed due to cosmetic purposes or complications. Thus, the observations are measurements of the effect from the first extensive reconstructive surgical procedure, independent of the duration of reconstructive surgery or the number of surgical events. Again, such subsequent procedures might represent a possible bias and the observed effects might therefore be diluted.

In conclusion, our study indicates that reconstructive breast cancer surgery constitutes an independent stimulating event on the growth of micrometastases leading to accelerated relapse rates. The effect is similar to that observed after primary breast cancer surgery. Importantly, this does not translate into worse long-term disease-free survival. Our results may provide indirect evidence that immediate reconstruction would be more beneficial than delayed as this obviates one possible growth stimulating event. Still, randomized trials assessing this question are not ethically or practically feasible. Further studies are ongoing and will shed more light on tumor biological mechanisms behind the observed phenomenon.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

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RESEARCH ARTICLE Open Access

Distant metastasis dynamics following subsequent surgeries after primary breast cancer removal

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Abstract

Background: The aim of the research was to separate the distant metastasis (DM) enhancing effect due to breast tumour removal from that due to surgical manoeuvre by itself.

Methods: DM dynamics following surgery for ipsilateral breast tumour recurrence (IBTR), contralateral breast cancer (CBC) and delayed reconstruction (REC), which was performed after the original breast cancer surgical removal, was analysed. A total of 338 patients with IBTR, 239 with CBC and 312 with REC were studied.

Results: The DM dynamics following IBTR, CBC and REC, when assessed with time origin at their surgical treatment, is similar to the analogous pattern following primary tumour removal, with a first major peak at about 18 months and a second lower one at about 5 years from surgery. The time span between primary tumour removal and the second surgery is influential on DM risk levels for IBTR and CBC patients, not for REC patients.

Conclusions: The role of breast tumour removal is different from the role of surgery by itself. Our findings suggest that the major effect of reconstructive surgery is microscopic metastasis acceleration, while breast tumour surgical removal (either primary or IBTR or CBC) involves both tumour homeostasis interruption and microscopic metastasis growth acceleration. The removal of a breast tumour would eliminate its homeostatic restrains on metastatic foci, thus allowing metastasis development, which, in turn, would be supported by the forwarding action of the mechanisms triggered by the surgical wounding.

Keywords: Breast cancer, Recurrence dynamics, Metastasis development, Second surgery, Tumour homeostasis, Surgery-related metastasis acceleration

Background

In the middle of the nineteenth century, the arguments set out by Virchow, who suggested that the disease starts as a single focus within the breast, then migrates to the axillary lymph nodes and ultimately to distant organs, supported the Halsted operation that was adopted as the default therapy worldwide [1]. However, among resected patients, 30% of node-negative and 75% of node-positive women still developed distant metastases and succumbed [2]. The failure of mastectomy and other more aggressive operations to cure patients and, moreover, novel biology-based assumptions on the disease course [3] suggesting that the extent of local treatment does not affect survival supported a reduction of the extent of surgery. Additionally, clinical investigations and mathematical modelling advocated that surgical resection might not always be beneficial [4, 5] providing evidence that, while it favourably modifies the natural history of breast cancer for the majority of patients, it may also hasten the metastatic development for a number of them, by triggering growth of occult tumour deposits. The concepts underlying this new model extended to the clinical level the results of a protracted history of investigations lasting more than a century [6].

Helpful hints about the new model were achieved from analyses of post-resection recurrence dynamics in early breast cancer patients undergoing potentially

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curative removal of the primary tumour [7, 8]. A model assuming post-surgery acceleration of disease progression by a burst of growth in previously dormant micrometastases appeared to best fit the clinical data. Similar findings were observed in non-small cell lung cancer [9]. While this acceleration apparently occurs at the time of local treatment, it is still not deciphered whether this effect can be ascribed to primary tumour removal (e.g. to removal of inhibitory factors) or to the surgical manoeuvre per se (e.g. CTC release, immune suppression and pro-angiogenic stimulus of wounding) or to both. This differentiation is important as it may open a window to new therapeutic approaches.

Clues about this subject may be detected by the analysis of the recurrence dynamics in patients who undergo subsequent breast surgical manoeuvres during the follow-up of the disease in addition to primary tumour surgical removal. Patients undergoing conservative surgery for their primary tumour may experience ipsilateral breast tumour recurrence (IBTR), and others may be diagnosed contralateral breast cancer (CBC) whatever the surgical approach for the primary tumour has been. Moreover, some patients undergoing mastectomy as the first surgical treatment call for breast reconstruction (REC) and undergo aesthetical surgery. We hypothesized different metastatic recurrence dynamics associated with the different surgical procedures due to the clinical presence (IBTR and CBC) or absence (REC) of a tumour reservoir in the breast. Therefore, we report here findings from the analysis of distant metastasis (DM) dynamics following IBTR, CBC and REC, which was carried out with the aim of unravelling the different roles, if any, of the two possible factors, i.e. breast tumour removal and surgical manoeuvre by itself.

Patients and methods

At the National Cancer Institute of Milan, three randomized clinical trials have been carried out in the past, investigating the role of different surgical approaches for primary tumour removal. Moreover, since preliminary results of the first trial on the breast-conserving treatment for early breast cancer, which provided evidence that conservative surgery plus chest wall radiotherapy was comparable to more aggressive resections [10], patients received breast-conserving treatment as routine practice outside randomized clinical trials (out-trial patients). All axillary node-positive (N+) patients were offered systemic adjuvant treatment with cyclophosphamide + methotrexate + fluorouracil (CMF) or CMF plus doxorubicin, while no further post-surgical systemic treatment was recommended to axillary node-negative (N−) patients. Adjuvant hormone therapy was not utilized within the randomized clinical trials and seldom employed for out-trial patients, as it was not considered mandatory at that time. Two other randomized clinical trials were accomplished on patients who, following mastectomy or breast-conserving treatment, were found to be axillary node positive (N+). Patients with one to three positive axillary lymph nodes were randomly allocated to receive either 12 courses of CMF or 8 courses of the same regimen followed by 4 courses of doxorubicin, while patients with > 3 positive axillary nodes were randomized to receive either four courses of doxorubicin followed by 8 courses of CMF or 2 courses of CMF and 1 course of doxorubicin for a total of 12 courses. All clinical data from patients enrolled into the reported clinical trials or treated outside of trials were systematically recorded and stored in standard format. Detailed descriptions of patients, treatments and follow-up modalities have been reported elsewhere [10–14]. In particular, data for patients suffering IBTR are reported in ref. [15].

A further database was analysed, including all breast cancer patients undergoing mastectomy who underwent delayed reconstructive surgery at Haukeland University Hospital, Bergen, Norway. The reconstructive procedures were implant surgery, implants combined with flaps, deep inferior epigastric perforator flaps and transverse rectus abdominis myo-cutaneous flaps. A paired control was randomly selected from patients with identical T and N stages, age ± 2 years, and follow-up without recurrence equal to or longer than the time to reconstruction of the respective matched reconstructed patient (defined as “reference time”, i.e. the time origin for the analysis of DM dynamics for controls). Patient characteristics and details of the study have been reported in ref. [16]. All studies supplying the analysed databases were approved by the institutional ethics committees and review boards in accordance with the Declaration of Helsinki.

The analysis of recurrence dynamics was focused on DM as the first event after the second surgery (the studied timing periods are outlined in Fig. 1): DM-free survival times were calculated as time elapsed since the second surgery (for IBTR, CBC or REC) to DM occurrence or to the last documented follow-up with no evidence of disease. Second primary tumours, including contralateral breast cancers, were considered as competing events, and the corresponding event-free survival times were censored at the time of their occurrence. The DM dynamics was studied by estimating with the life-table method the hazard rate for DM, i.e. the conditional probability of manifesting DM during a certain time span, given that the patient is clinically DM free at the beginning of the interval [17]. A discretization of the time axis in 6-month units was applied, and a Kernel-like smoothing procedure [18] was adopted.

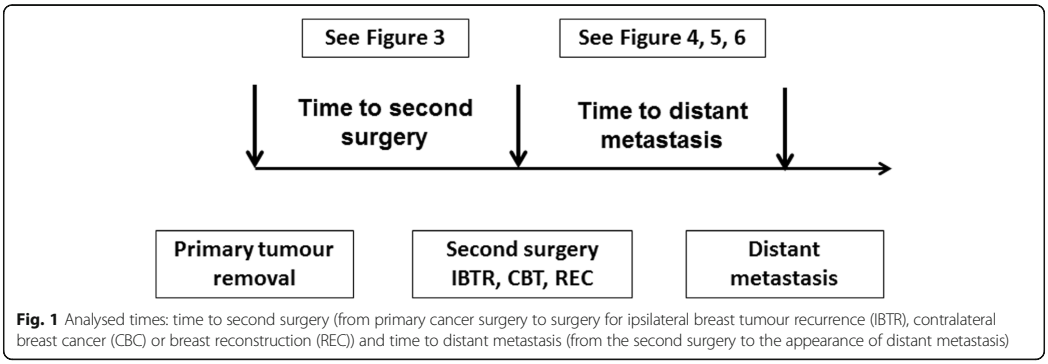

Fig. 1 Analysed times: time to second surgery (from primary cancer surgery to surgery for ipsilateral breast tumour recurrence (IBTR), contralateral breast cancer (CBC) or breast reconstruction (REC)) and time to distant metastasis (from the second surgery to the appearance of distant metastasis)

Results

A CONSORT diagram for IBTR, CBC and REC patients and matched controls is reported in Fig. 2.

Among patients undergoing conservative surgery, 92% received chest wall radiation therapy, mostly at the total dose of 50 Gy (daily dose 2 Gy) with high energy plus 10 Gy (daily dose 2 Gy) as a boost with orthovoltage to the ipsilateral breast. Following the diagnosis of IBTR or CBC, the treatment was decided on an individual basis. Median follow-up times after IBTR and after CBC were 151 months and 144 months, respectively. The number of patients suffering DM diagnosis within 10 years from IBTR and CBC was 138 and 84, respectively.

In the Norwegian study, the matched control group included patients who were extracted from a total of 868 (see the "Patients and Methods" section in ref. [16]). Median follow-up after reconstruction or reference time for controls was 137 months. The number of patients suffering DM within 10 years for REC and controls was 44 and 45, respectively.

Main patient characteristics at primary tumour treatment are reported in Table 1. In spite of the wide time span of patient accrual, the homogeneity of main prognostic factors across the databases, with the exception of tumour size in REC patients, is noteworthy. Axillary node involvement is near identical, as well as the frequency of ER-positive and ER-negative tumours among assessed ones, despite the fact that ER content was measured at different frequencies in the three series. Anyway, the DM dynamics of ER+ and ER− cancer have similar timing pattern [19] and, therefore, no modification of surgery effects on the time patterns was expected by oestrogen receptor levels. Moreover, as analysed patients suffering IBTR and CBC did not receive adjuvant endocrine therapy just like 42% of ER+ reconstructed patients, the question of whether endocrine therapy may alter DM patterns in a modern cohort remains open. HER2 status was not available and, accordingly, no specific treatment was administered.

The distribution of surgical treatments for IBTR, CBC or REC during the follow-up subsequent to primary tumour removal is reported in Fig. 3. Reconstructions were performed mainly during the first 5 years (median time 2.5 years) while IBTR removal had a more protracted distribution (median time 4.3 years) and a structured pattern [20]. CBC treatments have a steadier pattern consistent with the notion that the occurrence of a CBC may be considered a random event not time-related with primary tumour [21, 22].

The DM dynamics was analysed for the four groups in a timeframe with $t = 0$ at second surgery (Figs. 4 and 5 solid lines). Moreover, the influence of the time elapsed from primary tumour removal to the second surgical manoeuvre [time to second surgery] on the hazard rate for DM pattern was investigated as well (Figs. 4 and 5 dashed lines). The hazard rate pattern is similar for the three surgical groups with a first major peak at about 18 months and a second lower one at about 5 years from the second surgery, although the three levels of recurrence risk are different. Time to second surgery is apparently not influential for reconstructed patients, whereas it changes the first peak height for the other sets, showing that the influence is maximal for early re-operations, decreases afterwards and apparently disappears for time to second surgery values larger than 2 to 3 years. To ascertain whether factors known to be influent on the risk level may drive the described phenomenon, we analysed the DM dynamics by time to second surgery in IBTR patients pooled by axillary node status (node positive vs node negative) and by second surgery extent (mastectomy vs conservative surgery). In all analysed subsets, the time to second surgery aroused the same hazard rate pattern, as Fig. 5 exemplifies for the axillary nodal involvement. A comparison between REC patients and controls for DM hazard rate pattern is reported in Fig. 6, where the accelerating effect of surgery on the DM dynamics is quite evident.

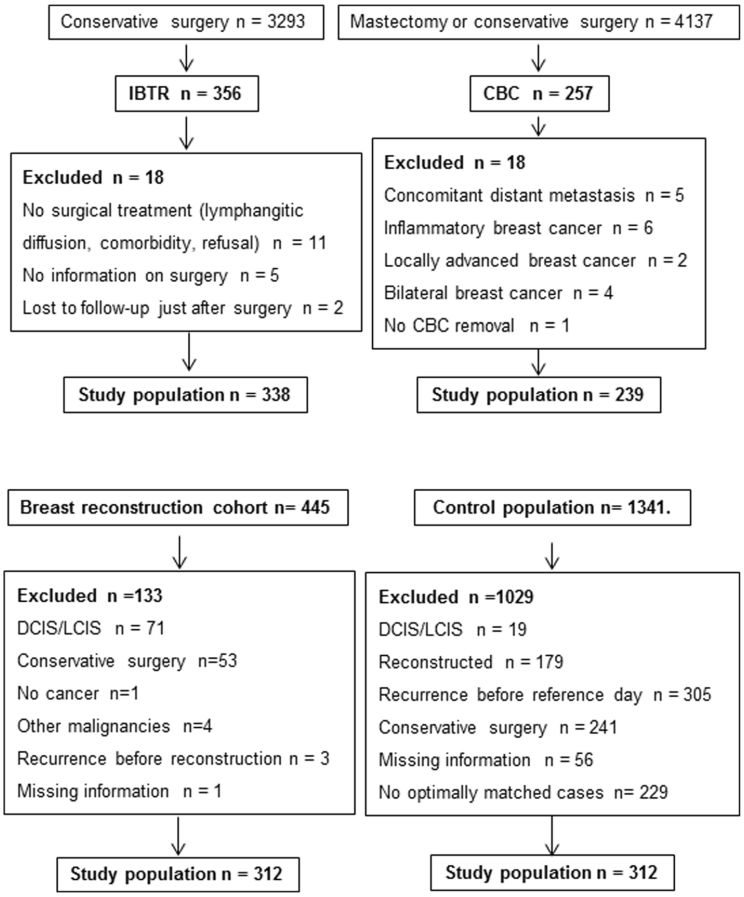

Fig. 2 A CONSORT diagram for IBTR, CBC, REC and control patients. Analysed data were from trials carried out between 1975 and 1990 (IBTR, CBC) and between 1977 and 2007 (REC, controls)

Discussion

Our analysis on breast cancer patients undergoing breast surgery for IBTR, CBC and REC provides two main results: (1) the DM dynamics following a new breast surgical manoeuvre performed after primary tumour removal is similar to the analogous dynamics following primary tumour removal and (2) the time span between the two operations is not associated with changes in peak timing; yet, for IBTR and CBC patients, it is related to progressive reduction of DM risk levels, while REC patients display similar DM risk levels for all time spans.

It should be emphasized that the three surgeries are performed in different clinical situations. Surgery for IBTR is strictly related to the specific multimodal dynamics of IBTR appearance [15] while CBC diagnosis and subsequent removal is an independent event with steady hazard rate [21, 22] and, finally, REC surgery is related to the patient's desire and only indirectly to clinical conditions. Therefore, when comparative analyses among patients with such different tumour-host settings at the time of surgery display similar behaviours, they are reasonably attributable to the act of treatment per se. Our findings suggest that there is a metastasis-enhancing effect in all surgical interventions, which displays, however, different traits related to whether a macroscopic breast tumour is removed or not.

The enhancing effect of surgical primary tumour removal on metastatic disease is well supported by a long history of investigations [6] and from a few clinical studies in humans (e.g. [23]). It enables to explain the

Table 1 Patient characteristics

	IBTR (338)	CBC (239)	Rec (312)	Controls (312)
Median age at diagnosis (years)	45	48	48	49
25%–75%	39–52	42–56	42–53	43–53
Range	21–69	22–75	29–73	28–71
Tumour size (%)				
T1	85	84	61	62
T2	12	15	29	30
T3/4	–	–	8	7
Missing	3	1	2	1
Node negative (%)	70	64	68	67
Node positive (%)	30	36	32	33
ER negative (%)	16	16	20	19
ER positive (%)	63	53	70	70
ER missing (%)	21	31	10	11

multipeak pattern of the hazard rate for recurrence in patients with early breast cancer undergoing surgery or surgery plus adjuvant chemotherapy [7]. Here, we confirm that this enhancing effect is observable even when surgical manoeuvres are performed in the breast area at a later date. The result of our analysis apparently diverges from the outcome of a previous investigation on the same subject [24] suggesting that traumas or intervening surgical procedures unrelated to cancer are not associated with an increased cumulative rate of breast cancer recurrence in a 2-year window. Although this discrepancy may be related to the shortness of the

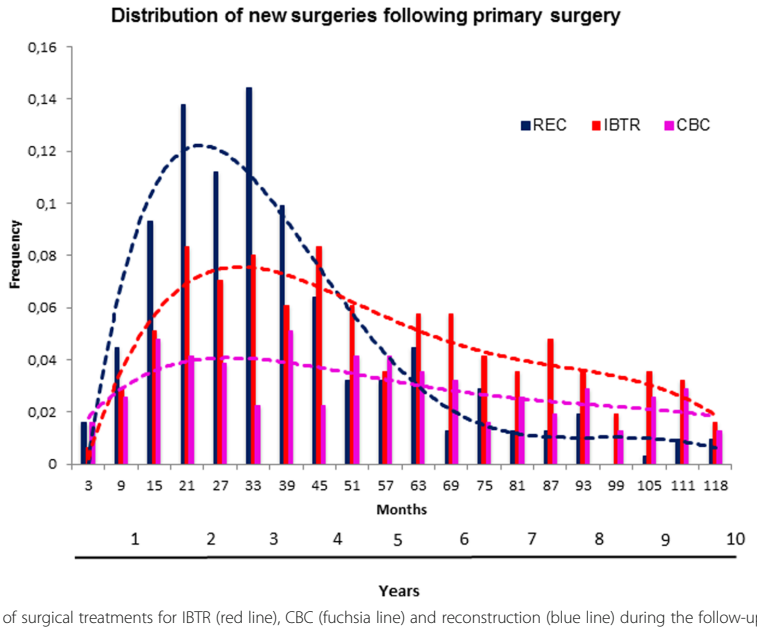

Fig. 3 Distribution of surgical treatments for IBTR (red line), CBC (fuchsia line) and reconstruction (blue line) during the follow-up with $t = 0$ at primary tumour removal. RECs were performed mainly during the first 5 years (median time 2.5 years) while IBTR removal had a more protracted distribution (median time 4.3 years) and CBCs had a steadier pattern consistent with the notion that the occurrence of a CBC may be considered a random event

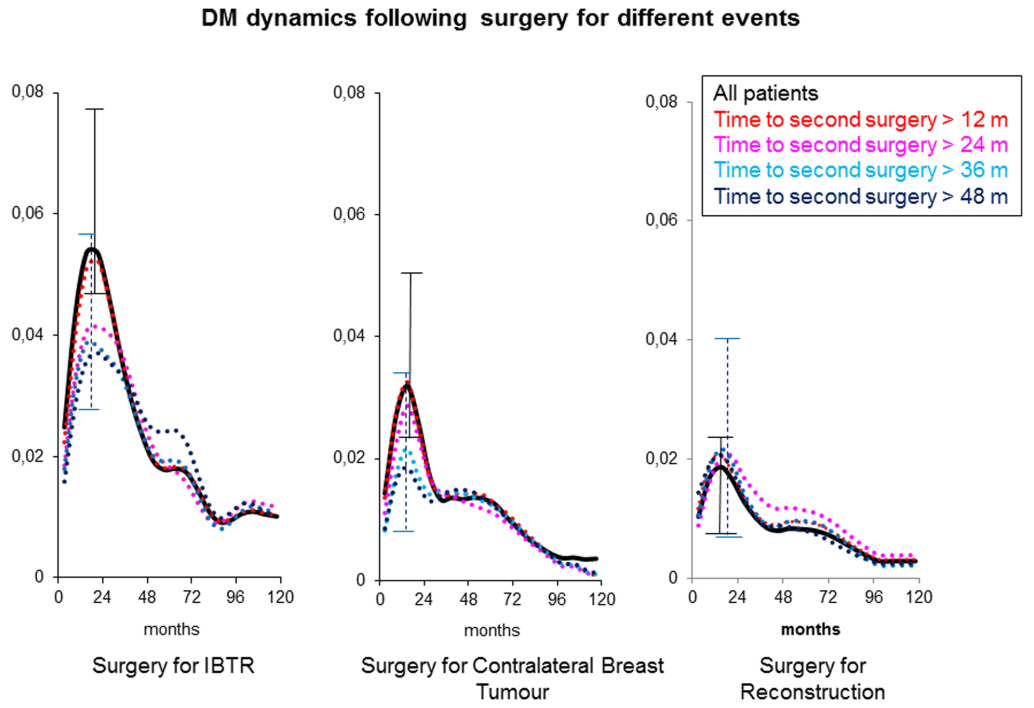

Fig. 4 Distant metastasis dynamics following surgery for IBTR, contralateral breast tumour and reconstruction ($t = 0$ at second surgery). The hazard rate pattern is similar for the three groups with a first major peak at about 24 months and a second lower one at about 5 years. The analysis was performed for all patients (bold lines) and, moreover, by time from primary tumour removal to second surgery as well (dashed lines). While this factor is not influent for reconstructed patients, it changes the first peak height for surgeries removing neoplastic nodules if the time to second surgery is less than about 3 years. Confidence intervals on the estimated hazard rates at the first peak position are reported for all patients and those with time to second surgery more than 48. *Y*-axis reports hazard rates for DM, i.e. the conditional probability of manifesting DM during an interval of 6 months, given that the patient is clinically DM free at the beginning of the interval. *X*-axis units are months between primary tumour removal and the second surgical manoeuvre

analysed interval, a subtler inference could be considered on the basis of the known topological specificity of cell populations [25, 26] and of the finding that, in an animal model, acceleration in tumour growth by mobilization of bone marrow-derived cells may be different after operative injuries to different organs [27]. Indeed, one could speculate that surgery in the breast area may stimulate distant breast cancer foci, unlike surgery in other sites. Unfortunately, this hypothesis cannot be disentangled since data on non-breast interventions were not available for comparison.

Of note, the hazard rate peaks, in particular the first one, have different heights in IBTR, CBC and REC (Fig. 4). This occurrence is in keeping with the notion that the sudden acceleration of metastasis development takes action on the underlying DM dynamics, which is different in IBTR, CBC and REC patients. Indeed, patients with IBTR have an intrinsic high risk of DM (similar to N+ patients) that was unpredictable by the usual prognostic factors at the initial treatment and that is revealed when IBTR emerges in advance of the competing DM events [15, 28]. In comparison with these patients, women suffering CBC display a considerably lower first hazard rate peak (about 60% peak to peak) in keeping with the concept that CBC is a second primary, unrelated to the first one [21, 29, 30]. Accordingly, patients with CBC actually fit to a population with "average" DM risk [31]. Finally, patients undergoing REC have better prognosis due to favourable selection criteria: they had no previous recurrence event and factors such as smoking, obesity and diabetes excluded patients from being offered complex breast reconstructive procedures. Moreover, the baseline risk in this population may be influenced by features here not accounted for, such as socioeconomic conditions, better general health and low body mass index, which is recently emerging as a prognostic factor in breast cancer [32].

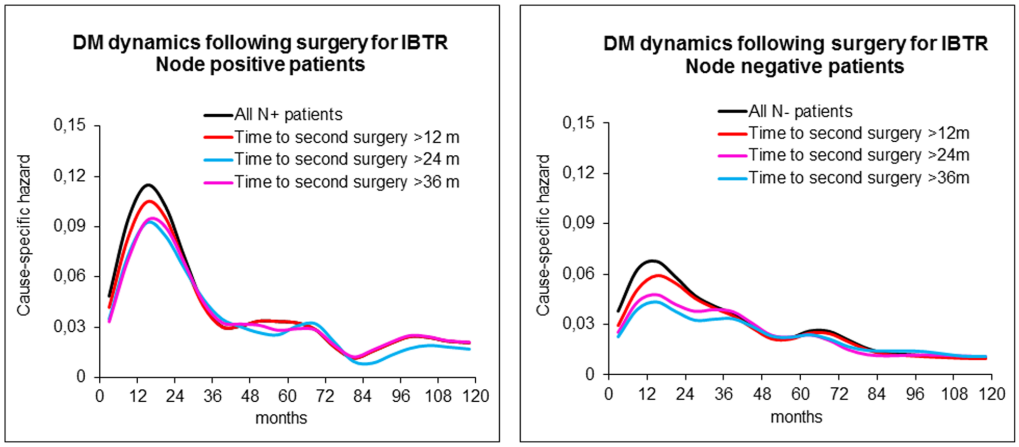

Fig. 5 Distant metastasis dynamics following surgery for IBTR ($t = 0$ at IBTR) in patients with axillary node positive (left) or negative (right). The analysis was performed for all patients and, moreover, by time from primary tumour removal to surgery for IBTR. While nodal status affects the risk level, which is higher for node-positive patients, the time to surgery for IBTR is still influential on the first peak height if it is less than about 3 years. *Y*-axis reports hazard rates for DM, i.e. the conditional probability of manifesting DM during an interval of 6 months, given that the patient is clinically DM free at the beginning of the interval. *X*-axis units are months between primary tumour removal and the second surgical manoeuvre

While there is a substantial body of evidence indicating that the surgery-associated tissue trauma and wound healing can promote growth, angiogenesis and metastatic ability of cancers [33], data on the possible homeostatic connection between primary tumour and its metastases are lacking. Our finding that the time to second surgery is influential on the risk level only for IBTR and CBC patients and not for REC patients suggests that tumour removal, which occurs in the former groups only, plays a specific role on metastasis development. This idea is in agreement with the recalled model of breast cancer metastatic development [7] if one takes into account that the manifestation of IBTR or CBC, i.e. of a macroscopic breast tumour, is preceded by a number of months of subclinical disease. According to the tumour homeostasis concept [7], during this time span, the growing tumour exerts constraints on distant microscopic foci, somehow mimicking the homeostatic processes underlying the control of size in adult organs and organisms [34, 35]. Although the molecular characteristics of these mechanisms are largely unknown, recent reports have provided initial interesting findings [36, 37], which may have oncological important implications as well [38].

In patients suffering IBTR or CBC, a number of metastatic foci are related to the previous breast cancer, although a few of them may be associated with the new breast neoplastic lump. The emerging restrictive interference results into some freeze of the microscopic metastases in the conditions existing when the new homeostatic action is starting. Taking into account the hazard rate dynamics for the DM related to the primary breast cancer [7], such a freeze should have effects depending on time to second surgery: the shorter this time, the higher the underlying DM risk. Consequently, while the DM dynamics after the IBTR or CBC removal maintains the usual time-related pattern, the corresponding hazard rate level would depend on time to second surgery. Following 2 to 3 years, the time to second surgery loses its prognostic value, in keeping with the drop of DM risk attributable to the primary breast cancer [7].

The finding that patients undergoing REC do not present any effect from the time to second surgery, while displaying the usual time-related pattern in the post-reconstruction DM dynamics, suggests that the reconstructive surgical manoeuvre, in the absence of any breast tumour removal, may act on metastasis development differently from IBRT and CBC surgical removal. As a working hypothesis, it may be assumed that surgical manoeuvres prominently act on the microenvironment of tumour foci turning it into conducive (e.g. by activating angiogenesis) and thus sustaining growth [39]. This facilitating action would simply speed up the clinical appearance of some metastases that would emerge later according to their own dynamics. This hypothesis is suggested by the comparison between the hazard rate patterns for DM in REC patients and in the matched paired control group (Fig. 6). This comparison suggests

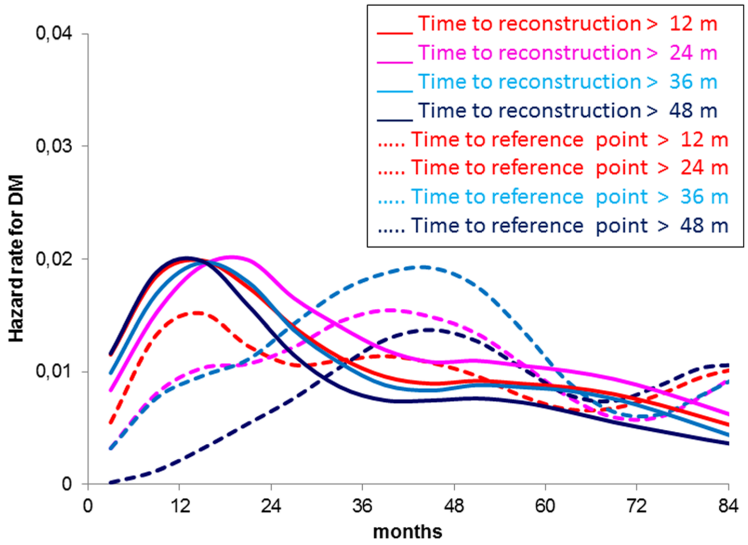

Fig. 6 DM dynamics for reconstructed patients and paired controls. Following surgery for reconstruction ($t = 0$ at second surgery), the hazard rate for DM displays a common pattern that is not dependent on the time from primary tumour removal to second surgery. On the contrary, the analogous analysis for controls ($t = 0$ at reference time, as defined in the "Patients and methods" section) displays a progressive change of the DM dynamics that is compatible with the time-related DM risk expected after primary tumour removal in the absence of the further surgical manoeuvre. *Y*-axis units are hazard rates for DM, i.e. the conditional probability of manifesting DM during an interval of 6 months, given that the patient is clinically DM free at the beginning of the interval. *X*-axis units are months between primary tumour removal and the second surgical manoeuvre

that the reconstruction is associated with a decrease of the hazard rate for DM at the fourth year and a concomitant increase of the hazard rate at the second year.

The present findings are coherent with and integrate the evidence coming from separate studies on IBTR and REC, resorting to advanced time scale statistical modelling [16, 40]. The overall picture provided support to the biological hypothesis, underlying the observed distant metastasis dynamics following surgeries performed after primary breast cancer surgical removal, according to different tumour homeostasis-related and surgical wound-related effects on metastasis development.

The analysed databases did not included data on HER2, preventing investigations on triple-negative patients. Moreover, we could not analyse the possible role of anaesthetic management, which potentially influences the long-term outcome most probably in patients undergoing more extended surgery [41], due to missing information about this factor.

Conclusions

In summary, the findings of the present analysis support the concept that the impact of breast tumour surgical removal (either primary or IBTR or CBC) on microscopic metastases is twofold, inasmuch as two different factors, i.e. tumour homeostasis interruption and surgical wound effects, are involved. The removal of a breast tumour would result into the sudden elimination of the restrains on metastatic foci, thus allowing metastasis development, which, in turn, would be supported by the forwarding action of the mechanisms triggered by the surgical wounding. This surgery-related phenomenon would underlie the behaviour of DMs in the REC group, where no detectable tumour deposit is removed. While associations of such latter phenomenon with surgical-related inflammatory conditions and different anaesthesia modalities are suggested from a few clinical data [41–43], the biological mechanisms underlying tumour homeostasis are largely unknown. Investigations in this field are urgently warranted.

Abbreviations

CBC: Contralateral breast cancer; CMF: Cyclophosphamide + methotrexate + fluorouracil; DM: Distant metastasis; IBTR: Ipsilateral breast tumour recurrence; N–: Axillary node negative; N+: Axillary node positive; REC: Delayed reconstruction

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Authors' contributions
RD contributed to the conceptualization, formal analysis, writing of the original draft and writing as well as reviewing and editing of the manuscript. HD and OS provided the resources and contributed to the writing as well as reviewing and editing of the manuscript. EB provided the resources and contributed to the formal analysis, writing of the original draft and writing as well as reviewing and editing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
All studies supplying the analysed databases were approved by the institutional ethics committees and review boards in accordance with the Declaration of Helsinki.

Consent for publication
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Competing interests
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IV

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