Monitoring of the *healthy* immune response in the sentinel lymph node of patients undergoing a prophylactic mastectomy.

Published: 04-07-2011
Last updated: 03-05-2024

Primary goal of this study is to establish the *healthy* immune response in SLN of patients undergoing a prophylactic mastectomy.Secondary goal is to determine the incidence of IHC-positive SLN due to *benign* epithelial cell displacement

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Breast neoplasms malignant and unspecified (incl nipple)
Study type	Observational non invasive

Summary

ID

NL-OMON36250

Source
ToetsingOnline

Brief title
Immune responses in the sentinel lymph node.

Condition

- Breast neoplasms malignant and unspecified (incl nipple)

Synonym
Breastcancer, mammacarcinoma

Research involving
Human

Sponsors and support

Primary sponsor: Antoni van Leeuwenhoek Ziekenhuis

Source(s) of monetary or material Support: Posthumus Meyjes Fonds

Intervention

Keyword: Breastcancer, Immune response, Sentinel lymph node

Outcome measures

Primary outcome

Establishing the *healthy* immune response in SLN of patients undergoing a

prophylactic mastectomy.

Secondary outcome

NA

Study description

Background summary

With a life time risk of 12-13 percent, breast cancer is one of the most common forms of cancer among women. For the locoregional treatment of breast cancer, the sentinel lymph node biopsy (SLNB) has become a standard diagnostic procedure for accurate nodal staging. Because of the typical lymphatic drainage pattern from the breast to the axilla, the sentinel lymph node (SLN) is the first lymph node in the axilla to drain the primary tumor and therefore the site with the highest chance of metastatic involvement and subsequent activation of the immune system. For this reason the SLN has become an increasingly popular focus for tumor immunological research.
However, sometimes the immune system fails to fight off cancer cells leading to metastasis.
Several mechanisms are responsible for this so called tumor escape. From earlier melanoma research we now know that immune suppression is strongest in and around the primary tumor and in the surrounding lymph nodes, especially in the SLN1,2. Evidence suggests that dysfunctional dendritic cells and immune suppressive regulatory T-cells (Treg) play a major role in this process3,4 In breast cancer, it is still unknown exactly how this immune suppression is established and how it is expressed on a cellular level.

For accurate monitoring of possible functional and quantitative immunological changes in the SLN of breast cancer patients it is crucial to understand how a normal immune response in SLN of healthy patients ensues. For this purpose, SLN

of patients undergoing prophylactic breast surgery, in our opinion, can provide reliable information regarding a normal immune response. This normal immune response can be used as baseline measurement for further immunological research in breast cancer SLN.

Every year, The Netherlands Cancer Institute performs a large number (20-25) of prophylactic mastectomies. This large group of patients from the NKI combined with experience from our research group (KG/ VUmc Cancer Center) provides a extremely suitable combination for immunological studies of SLN in healthy patients.

Besides the fact that SLN has become a popular focus for tumour immunological research, the increased use of the sentinel lymph node biopsy in breast cancer has lead to a discussion concerning the discovery of false positive findings in routine pathological examination. Although SLN biopsy allows enhanced analysis of lymph nodes by serial sectioning and staining by immunohistochemistry (IHC), there is controversy regarding the clinical significance of IHC-detected, cytokeratin-positive cells in SLN, also classified as isolated tumor cells. A number of studies describe iatrogenic epithelial cell displacement and benign transport of epithelial cells to axillary lymphe nodes, caused by mechanical means such as needle biopsies or even manipulation of the breast during surgery.
IHC is performed with antibodies that are epithelial cell specific and not breast cancer specific, therefore IHC cannot distinguish between these potentially displaced *benign* epithelial cells and true metastases.
From this point of view, SLN from patients undergoing prophylactic mastectomy offer a unique possibility to examine the incidence of IHC-positive SLN due to *benign* epithelial cell displacement. Because patients undergoing prophylactic mastectomy do not have breast cancer, we can exclude the possibility of true metastases when IHC-positive cells are detected.

Sentinel lymphe node and lymphatic drainage pattern of the breast
In breast cancer, the primary lymphatic drainage pathway for tumor cells is to the axilla. The existence of lymphatic drainage of the breast to extra-axillary sites has been known for centuries. Although this may result in better staging, the presence of these extra axillary nodes have no consequences in the standard treatment of breast cancer patients5. In breast cancer (T1-2N0), the sentinel lymph node biopsy (SLNB) is used as a primary lymph node staging procedure. The SLNB is most reliable when performed by the triple technique, using preoperative lymphoscintigraphy and periareolar injection of blue dye (Patent blue), followed by γ-probe-guided surgery6. Serveral studies report that in more than 95% of all patients a sentinel lymph node can be found and that in 95% of the patients this SLN can accurately predict absence or presence of additional axillary lymph node metastasis7-11.

In case of prophylactic mastectomy the sentinel lymph node biopsy is not part

of the routine procedure. In order to avoid additional burden for patients participating in this study a pre operative lymphoscintigraphy will not be performed. For identification of the SLN Patent blue will be used, but unlike the standard procedure we will inject the blue dye into the breast parenchyma, below the subcutaneous fat, to avoid tattooing the overlying skin.
Based on the fact that also in healthy patients the typical lymph drainage pathway is to the axilla, we believe that these SLN provide the most reliable information regarding a normal immune response. This normal immune response can be used as baseline measurement for further immunological research in breast cancer SLN.

Immune respons in the SLN in breast cancer
Our immune system can play an important role in the anti-tumor response. This anti tumor response is initiated through the same channels as a normal immune response. Tumor infiltrating dendritic cells (DC) take up and engulf antigens from the tumor. Once migrated to tumor draining lymph nodes (TDLN), DC present these antigens to CD4+ T-helper (Th) and to CD8+ Cytotoxic T-Lymfocytes (CTL) that are capable of initiating a specific anti-tumor immune response. It is however known that tumors can escape this immune surveillance leading to metastasis. Several mechanisms are responsible for this so called immune escape: immunosuppressive factors in the tumor environment, dysfunctional T cells that are incapable to penetrate the tumor, loss or downregulation of co-stimulatory molecules on DC which causes T cell anergy or immune suppression by suppressive regulatory T-cells (Treg)3.

Several studies have indentified immunological alterations in SLN of breast cancer patients. Some authors describe breast cancer SLN as immune competent, with increased presence of (mature) DC, prior to the process of metastasis12-14. Others suggest more severe immunosuppression in SLN compared to non SLN, prior to metastasis15,16. Thus, to date, it is still unclear when this immunosuppression in breast cancer SLN ensues, how it exactly affects the activation state and functionality of the different DC and T-cel subsets, and what the underlying mechanisms are. Therefore, additional immunological research is necessary.

To our knowledge, there have been no studies describing the immune respons in axillary SLN of healthy patients. For accurate monitoring of possible functional and quantitative immunological changes in the SLN of breast cancer patients it is crucial to understand how a normal immune response in SLN of healthy patients ensues. The aim of this study is to establish this healthy immune respons.
In another study, initiated by our research group and running parallel to this project, the immune response in SLN of breast cancer patients will be established by functional and quantitative immunological analyses.

Detection of isolated tumor cells
Presence of axillary metastasis is one of the most important prognostic criteria in breast cancer. As described earlier, the SLNB is an established method to predict axillary metastasis. Detailed examination of the sentinel node by means of serial sectioning with optional immunohistochemical staining permits the detection of small metastases or isolated tumor cells (ICT). In 2002, the American Joint Committee on Cancer (AJCC) revised the breast cancer staging system and classify micro-metastasis (deposits >0.2 to <=2.0 mm) and ICT (with deposits <=0.2 mm) as separate categories next to macro metastasis17. Regarding the clinical significance of detected micro-metastasis, several studies have shown that the risk of additional axillary lymph node involvement is up to 15%. Furthermore, SLN micrometastasis are associated with a worse prognosis18-20. Therefore, additional axillary lymph node dissection (ALND) is warranted and adjuvant treatment with chemotherapy should be considered.
In case of isolated tumor cells detected in SLN, the clinical and prognostic significance is still in question. Reed et al. and Cox et al. 21,22 report that isolated tumor cells do no influence disease free survival in their series of patients. On the contrary, authors of the recently published MIRROR study, conclude that ICT in SLN were associated with a reduced 5-year disease free survival rate, among women with favourable early-stage breast cancer who did not receive adjuvant therapy. Interestingly, the observed prognostic significance of ICT was similar to that of micro-metastasis23. The current Dutch guidelines regarding the treatment of breast cancer do not recommend ALND for patients with ICT positive SLN.

Pathological examination of SLN includes hematoxylin and eosin (H&E) step sections and more enhanced pathologic analysis with immunohistochemical stainings (IHC) for detection of micrometastasis or ICT that are not found on H&E examination. IHC is performed with the cytokeratin antibodies CAM 5.2 and/or AE1/AE3. IHC methodology employs antibodies that are epithelial cell specific and not breast cancer specific.
It is known in literature, that false positive findings can occur using the IHC methodology and that ICT detected by IHC may not be true metastasis. Several studies have suggested that IHC-detected cells in SLNs of patients with breast carcinoma represent mechanically displaced benign or malignant epithelial cells resulting from manipulation of the breast during mammography, SLN biopsy or surgery. It has also been described that pre-operative needle biopsy or needle localization, can displace epithelial fragments into nearby lymphatic channels, which result in transportation of epithelial cells to the axillary lymph nodes. This so called *benign transport* is thought to be responsible for *falsely positive* detected ICT in SLN24-27. Furthermore, there seems to be evidence that some IHC detected ICT concern tumor cells with limited malignant potential, which likely lack outgrowth potential and therefore not clinically relevant28.

Most of the previous studies examining the incidence of IHC-positive cells in SLN have evaluated patients with breast cancer, making it difficult to exclude

the possibility of true metastases when cells are positive on IHC staining. Because in this study only SLN of patients undergoing PM (without cancer) are examined, these SLN should not contain metastasis of tumor cells. However, owing to the surgery itself, mechanical manipulation of the breast does occur. Thus, when IHC positive cells are detected in these SLN, we can exclude the possibility of true metastasis and confirm the hypothesis that iatrogenic displacement of benign epithelial cells causes false positive findings.

Study objective

Primary goal of this study is to establish the *healthy* immune response in SLN of patients undergoing a prophylactic mastectomy.
Secondary goal is to determine the incidence of IHC-positive SLN due to *benign* epithelial cell displacement

Study design

From 20 patients undergoing a prophylactic mastectomy, the SLN will be removed during the same surgery. Normally the sentinel node is identified by the triple technique using preoperative lymphoscintigraphy and periareolar injection of blue dye (Patent blue), followed by γ-probe-guided surgery. In this study however, patients are included who undergo prophylactic mastectomy and the sentinel lymph node biopsy is not part of the routine procedure. In order to avoid additional burden for patients participating in this study a pre operative lymphoscintigraphy will not be performed. For identification of the SLN Patent blue will be used, but unlike the standard procedure we will inject the blue dye into the breast parenchyma, below the subcutaneous fat, to avoid tattooing the overlying skin.
The removed SLN will be bisected crosswise. Half will be used for immunological analysis and the other half for pathological analysis by serial sectioning and staining by immunohistochemistry (IHC).

Study burden and risks

Patient will undergo an additional sentinel lymph node procedure. This extends the operationtime with approximately 15 minutes. Furthermore, due to the injection of Patent Blue, which is part of the sentinel lymph node procedure, patients might get an anafylactic reaction.

Contacts

Public
Antoni van Leeuwenhoek Ziekenhuis

Plesmanlaan 121
1066 CX Amsterdam
NL
Scientific
Antoni van Leeuwenhoek Ziekenhuis

Plesmanlaan 121
1066 CX Amsterdam
NL

Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Women
Age 18 yrs or older
Undergoing prophylactic mastectomy
Written informed consent

Exclusion criteria

1. Presence of invasive breast cancer or ductal carcinoma in situ upon pathological examination
2. Contralateral breastcancer
3. Any other form of cancer
4. Any surgery or (needle) biopsy of the breast or axilla in the last 3 months
5. Current immunosuppressive therapy (including chemotherapy)
6. Current immunotherapy
7. Autoimmune disorders

Study design

Design

Study type: Observational non invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Other

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	31-01-2012
Enrollment:	20
Type:	Actual

Ethics review

Approved WMO

Date:	04-07-2011
Application type:	First submission
Review commission:	PTC Stichting het Nederlands Kanker Instituut - Antoni van Leeuwenhoekziekenhuis (Amsterdam)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL34989.031.10