Detection of Tumour Containing Sentinel Lymph Node in Breast Cancer by Injection of Fluorescence Tracer through "Dual Route" in Breast Tissue and Intravenously

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Abstract: A new technique of identifying tumour containing sentinel lymph node has been developed. Sentinel node biopsy is the current standard of care practice for assessing axillary nodal status in ladies with early breast cancer. Sentinel node biopsy is performed by using a combination of a blue dye and an isotope tagged to a large particulate matter like sulphur colloid or albumin. These tracers are injected in the breast tissue or skin over the breast. We describe a new method of "dual route of injection" of tracers both in the breast as well as in the systemic circulation through a peripheral vein. This method of dual route injection is expected to detect the axillary nodes containing tumour metastasis and reduce the probability of missing a "false negative sentinel lymph node".

1 INTRODUCTION

Assessment of axillary nodal is necessary for proper staging and planning adjuvant therapy and extent of surgery in patient with breast cancer. Traditionally this was done by full axillary node dissection. Axillary lymph node dissection leads to morbidity such as edema in arm, pain, and shoulder stiffness. Hence, sentinel node biopsy has been developed as a minimally invasive procedure for axillary lymph node evaluation. In sentinel node biopsy surgeon removes the first lymph node that receives lymphatic drainage from the tumor. Therefore, sentinel node biopsy is associated with minimal morbidity (Schrenk, 2000). Currently, Sentinel node biopsy is considered the "standard of care" method for axillary nodal evaluation. Among women with early breast cancer with a clinically negative axilla, the sentinel node biopsy will identify about one third women who will have sentinel lymph node metastasis. In the largest randomized controlled trial of sentinel node biopsy, the NSABP- B32 trial (Krag, 2010), sentinel lymph node metastasis was detected only in 29% women. The sentinel node biopsy is carried out by injecting a blue dye and isotope in the diseased breast in the skin, deep to areola, or in and around the tumor. This route of injection detects a sentinel node with about 95% identification rate and a false negative node rate of 5% to 10% (Hiram 2006).

In this proposal we describe a new method of identifying a lymph node harboring tumor deposits. It is expected that the use of **dual route** of injection of tracers will reduce the probability of missing a false negative sentinel lymph node.

Kawada and Taketo (Kawada, 2011) have described the mechanism of metastasis to a lymph node. The metastasis in the lymph node is classified as a "macrometastasis" if it measures more than 2 mm in diameter on histological section (Takeuchi and Kitagawa). A metastasis is described as "micrometastasis" if it measures between 0.2 mm to 2 mm in diameter. The presences of only few tumour cells in the lymph node are called "isolated tumour cells". Long term studies in women with early breast cancer have revealed that presence of

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isolated tumour cells and micrometastasis in a lymph node do not increase the chance of locoregional or systemic recurrence and do not adversely affect disease free survival or overall survival. However, presence of a "macrometastasis" in a lymph node is determinant of increased locoregional failure. Thus, from a treatment point of view, it is important to detect an axillary lymph node containing a macrometastasis of > 2 mm in diameter.

Studies on animal and human tumors have demonstrated that tumor growth is dependent on angiogenesis (Kahlert 2014) . After, initial growth to a diameter of 0.2-2 mm, Tumor cells begins to secrete "Vascular endothelial growth factors" - VEGF family (VEGF-A, B, C & D) (Robert 2007). The sentinel lymph node containing the metastasis of more than 1 mm will possess angiogenesis induced by the malignant cells. This neovascular network of blood vessels will increase the blood flow in the lymph node containing a tumour deposit of >1 mm diameter. This increased blood flow can be detected by Doppler ultrasound flowmetry. The increased blood flow in these lymph nodes will allow higher concentration of intravenously injected fluorescein or other tracer.

2 RESEARCH HYPOTHESIS

1. Macro-metastasis in a lymph node is associated with tumor induced angiogenesis.

2. This angiogenesis would result in increased blood flow to the node harboring a macrometastasis.

3. Increased blood flow can be detected by Color Duplex Ultrasound scan with Power Pulsed Doppler flow-metry and intravenous injection of Fluorescein.

4. Fluorescein containing lymph node can be detected by fluorescence when viewed with blue light during sentinel node biopsy.

2.1 Background

One of the authors (Anurag Srivastava) had earlier described the presence of increased blood flow in skin melanomas of thickness greater than one millimeter (Srivastava, 1988). The characteristics of "Doppler frequency shift signals" are predictive of 15 year outcome of patient with skin melanoma (Srivastava, 2012). One advantage of this method of detection of sentinel lymph node would be that it will only detect nodes harboring a macro-metastasis. Since, angiogenesis sets in at a size of 1-2 mm tumor size, smaller micro-metastasis and isolated tumor cells will not be detected by this method. The presence of micro-metastasis and isolated tumor cells in the sentinel lymph node does not adversely effect the disease free survival and does not necessitate any further axillary therapy (axillary lymph node dissection or axillary radiotherapy).

Fluorescein angiography has been successfully utilized in the detection of tumor metastasis (Aalders, 2001) in ovarian tumours.

3 PROPOSED METHOD

Patients presenting with early breast cancer with a no palpable nodes in the axilla, will be requested to take part in this study.

Preoperative sonographic imaging axilla will be carried out to detect any suspicious node which will be biopsied. Women with a positive node biopsy will be treated with axillary lymph node dissection along with breast surgery. The patients without a sonographically suspicious node and those with a negative node biopsy report, will be offered a sentinel node biopsy and will be selected for the present study.

3.1 Method of Sentinel Node Biopsy and Fluorescein

3ml of 20% fluorescein sodium will be injected intravenously about 20-25 minutes before the sentinel node biopsy. It is assumed that fluorescein will reach the tissues and lymph node in this period and sentinel lymph node containing the metastasis of more than 1 mm would demonstrate higher concentration of fluorescein.

Usual sentinel node biopsy will also be carried out employing technitium tagged sulphur colloid and indocyanine green intradermal injection in periareolar skin of breast. Indocyanine green has the advantage of depicting the lymphatics and fluorescent node through the skin; hence a very small incision will be sufficient for location of sentinel lymph node. Indocyanine green induced fluorescence will be examined with a Near Infrared light (of 800 nm wavelength).

Sentinel Node Biopsy will be carried out under general anesthesia. Fluorescent and radioactive hot nodes will be removed and sent for histological examination. Intravenous Fluorescein should demonstrate lymph nodes containing tumour cells with a deposit of greater than 1mm. These fluorescein containing nodes will be sent separately for histological examination.

The fluorescent nodes and primary breast tumour

after removal during surgery are being bisected and smears are being prepared on a glass slide for imprint cytology. The smears are also being prepared on "Half reflecting slides " and being examined under the "Interference Microscope" at Department of Optics; Indian Institute of Technology -Delhi .

3.2 Observations

We applied this technique on two patients. In these patients we injected fluorescein sodium 3ml intravenous injection (IV) 20 min before incision.

We found highly fluorescent nodes in the axilla of these patients. Histologically these nodes were found to contain metastasis from breast cancer. Fluorescent node in the axilla can be seen in Figures 1 and Figure 2 shows fluorescent node separately.

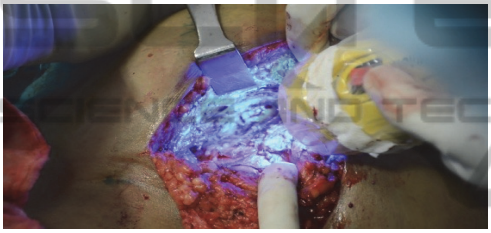

Figure 1: Axilla of breast cancer patient showing fluorescent node.

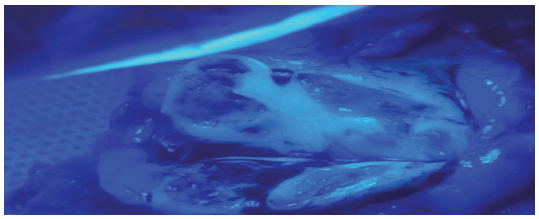

Figure 2: Fluorescent Node with Fluorescein injected IV.

4 DISCUSSION

Tumor induced angiogenesis has been demonstrated in many tumors. The onset of angiogenesis ushers in a phase of rapid growth, invasion into tissues and metastasis. Since most sentinel Nodes have been shown to harbor macrometastases (larger than 2 mm) or micrometastases (0.2 to 2mm), the growth of cancer cells inside a lymph node should be associated with neo-vascularisation. Our work in skin melanoma (Srivatava, 1988) has demonstrated that neovascularisation in tumour growth is an early event and can be detected in as early as 1mm thick melanoma. This was the first demonstration of angiogenesis in a clinical setting and Dr Judha Folkman wrote an editorial on this finding (Folkman, 1987). The tumour vasculature differs from normal vessels in being devoid of smooth muscles and precapillary sphincters and having large diameter vascular network with many arteriovenous communications. The blood flows through this vascular network at a high velocity with low impedance.

In this paper we propose the application of systemic administration of Fluorescein sodium intravenously, 20 to 25 min before incision. This will facilitate detection of tracer in the primary tumour as well as in the metastatic lymph node. It is hypothesized that the increased blood flow through a leaky vascular network in the tumour and metastatic node would enable high concentration of fluorescein or other tracer.

Current techniques of sentinel node biopsy involve injection of a blue dye and an isotope tagged to a large particulate material viz. sulphur colloid or albumbin in the breast tissue or skin over the breast. These tracers pass through the lymphatics of the skin or breast parenchyma and reach the lymph node (s) in the axilla. Sometimes, the lymphatics may get blocked by tumour cells or fibrosis due to prior surgery, inflammation or radiotherapy. In these patients the tracer does not reach the lymph node containing tumour metastasis. Instead, the tracer dye spreads to some “other lymph node” in the axilla, which does not contain tumour cells. If these “other lymph node(s)” are removed and sampled as “sentinel lymph node”, they are reported by pathologist as negative for cancer metastasis. However, the cancer metastasis containing lymph node is not identified because its lymphatics are blocked. Such a lymph node missed by present technique of injecting dye in breast is called the “False Negative sentinel lymph node”. The occurrence of “false negative sentinel lymph node” is reported in 5 % to 10% of patient with early breast cancer. In these patients this “false negative sentinel lymph node” is left behind in the axilla and may grow to present as an axillary lymph node recurrence later in life.

Therefore, we need to develop a method of detecting this “false negative sentinel lymph node”.

Our proposed method of injection of tracers through the “**dual route**” both through the tissue in breast as well as intravenously will detect the tumour containing lymph node and reduce the probability of a missing “false negative sentinel lymph node”.

Primary tumour in the breast is treated by wide

local excision. If tumour is resected with minimal tissue around it there is chance of leaving tumour cells in the breast. This may result in recurrence in the breast. Therefore, it is mandatory to remove the tumour with negative margins. Our proposed method will demonstrate a zone of increase fluorescence around the tumour because tumour vasculature is most abundant at the periphery of the tumour. Hence, an excision of tumour including the zone of increase fluorescence would accomplish complete tumour ablation.

5 CONCLUSIONS AND FUTURE DIRECTION

The proposed method is expected to detect cancer containing sentinel node thus reducing the probability of false negative rate. If the proposed methodology is able to detect positive lymph node with very high accuracy then we may refine the technique of sentinel node biopsy only to intravenous injection of the tracer because our objective is to detect malignant lymph node.

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