**Axillary Web Syndrome: evidence for lymphatic origin with thrombosis**

Running: Axillary Web Syndrome

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**ABSTRACT**

Background: The axillary web syndrome (AWS) occurs in the axilla and on the frontal side of the upper arm and sometimes along the forearm to the thumb. The cord is painful, particularly on movement, and can therefore be very distressing for the patient. Although the phenomenon has been examined and discussed for decades, no evidence for the origin has been found until now. The aim of this study was to perform a histopathologic analysis of cords taken 1996-98 in the Surgical Clinic, Skane University Hospital, Lund, Sweden.

Methods and Results: In seven patients, biopsies of the AWS cords were obtained 4-5 weeks after axillary node surgery for breast cancer and examined with standard H&E and D2-40 (lymphatic endothelial cell) staining. In one biopsy, there was a dilated vessel with a thickened wall which was confirmed by D2-40 immunostaining to represent a lymphatic vessel. The lumen was occluded by organised thrombus, within which new vessels were being formed, indicating recanalisation. In two other biopsies, similar lymphatic vessels with thickened walls were present although the lumen of the vessels were not visualised in the planes of section. The other four biopsies do not show specific features.

Conclusion: Although only one case, this is the first pathological evidence of thrombosis within a confirmed lymphatic vessel from a case of cording. We propose that the axillary cord represents lymphatic vessel thrombosis. Recanalisation of the thrombus may eventually restore lymphatic flow consistent with the transient nature of the condition.

Keywords: lymphatic thrombosis, axillary cord, breast cancer, pain

**INTRODUCTION**

The axillary web syndrome (AWS), otherwise known axillary cording or lymphatic cording, is a common occurrence after breast cancer treatment, but its pathophysiology has not been understood. Here, we present evidence that it is of lymphatic origin and likely due to lymphatic thrombosis.

The phenomenon can be visible in the axilla as soon as two weeks after axillary node dissection, usually for cancer. Although predominantly observed in the axilla, the cords can often be seen or palpated on the medial side of the upper arm and may extend down into the forearm turning laterally towards the thumb. Cords, like violin strings, are aligned in the direction of the neurovascular bundle. Tension on the cords through abduction of the arm induces pain and the cords can be seen bowstringing across the axilla so lifting the overlying skin, hence the term axillary web syndrome.

The pain can limit the range of motion (ROM) of the shoulder. In a prospective, longitudinal study by Johansson et al. (1), cords could be palpated in 77% of cases one month after axillary node dissection. The shoulder ROM was reduced in 66% and 43%, at examination one and two~~,~~ months post-surgery, respectively. However, at three months post-surgery the frequency for reduced ROM had decreased to 3 %. This means that the axillary cord is a minor problem in the long term. However, the reduced shoulder ROM and the pain by movement can be very distressing to the patient and may also cause problems with positioning of the arm for radiotherapy. Management varies from manual snapping of the cords to leaving alone for natural resolution. Symptoms can persist for 6 months or more (2). Doubt remains if AWS can predispose to lymphoedema (3).

If the cause of the cord could be determined, a proper treatment could be suggested as, at the moment, many different approaches are applied, often with contradictory background theories.

In a systematic review, Yeung et al (4) found that biopsies had identified venous or lymphatic pathology, but discrimination between the two vessels was often not possible (1, 5-7). Thrombosis of a presumed lymphatic vessel was reported in three studies (5-7), where identification of the type of involved vessel was based only on morphology. Lymphatic endothelial antibodies for lymphatic identity now exist making distinction between small veins and lymphatics possible (8). Here we report on findings in 7 specimens of cording using standard H&E histopathology and immunocytochemistry to determine the lymphatic origin of the cord**.**

**MATERIAL AND METHODS**

**Patients**

Biopsies from seven patients with axillary cord were taken in 1996-98. At that time, it had not been possible to distinguish clearly between veins and lymphatic vessels and the results showed a mixture of features (1). The seven patients with AWS included six female and one male treated for breast cancer (Table I).

Patients were informed about the biopsy procedure and gave their oral permission that was noted in their records. Ethical approval was obtained (Dnr 2016/501) with approved amendment for further sections to be cut and stained from the original tissue blocks 2018-03-26, from the Regional Ethical Review Board, Lund University, Sweden. The archived tissues imbedded in paraffin blocks were requested from Region Skåne biobank Department of Clinical Genetics and pathology Skåne County after permission from Regional Biobank centrum South Sweden. The tissue biopsies samples were transferred for analysis at the St George’s Hospital, London, UK. after approval from the Swedish Biobank centrum South Sweden.

**Procedure**

Four to five weeks post breast cancer surgery and with the patient in a supine position, passive abduction of the shoulder on the operated side was measured with a goniometer. The ROM was expressed in degrees as recommended by the American Academy of Orthopedic Surgeons (9). Once the cords were identified by palpation, local anaesthetic was infiltrated. Through a 2 cm long cut over the hard, palpable cord, 1-2 cm of the cord were ligated and taken out with some connective tissue around the cord. The biopsies were fixed in formalin and embedded in paraffin at the Clinical Pathology Unit, Lund, Sweden according to the clinical standards of tissue processing.

Fixation time of the tissue cannot be exactly reported as fixation time varied depending on tissue type, variable transfer time to the Pathology Unit and processing method. Tissue embedded in paraffin and archived in biobanks at room temperature shrink and dry out with the passage of time.

**Immunohistochemistry**

Formalin-fixed paraffin-embedded tissue blocks were cut on Leica RM 2250 microtome. The tissue was cut at three-micron-thickness (Leica microtome nr.2255) slides. The slides were pre-treated using heat-induced antigen retrieval with high pH (EnVision FLEX Target Retrieval Solution, K8010, Dako, Glostrup, Denmark) in a Dako Pre-Treatment Module. Immunohistochemical stainings were carried out in a Dako Autostainer Plus (DakoCytomatio, Glostrup, Denmark).

Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 10 min. The slide sections were then incubated for 1 h with rabbit polyclonal antibodies directed against Prox1 (1:300, ab38692, Abcam, Cambridge, UK) or mouse monoclonal antibodies against Ki-67 (1:300, M7240, Dako, Glostrup, Denmark). Staining was visualized using Polymer/HRP-linked secondary antibodies (K8010, Dako) and 3,3’-diaminobenzidine (DAB) chromogen (brown-colored product, SK-4100, Vector Laboratories, Burlingame, CA, USA). Next, the slide sections were treated with Denaturating Solution kit (DNS001L, Biocare Medical, Concord, CA, USA) for 5 min to prevent additional binding to the first primary antibody (10,11). The slide sections were then incubated for 1 h with mouse monoclonal antibodies against D2-40 (1:25, CM266, Biocare Medical) and staining was visualized using Polymer/HRP-linked secondary antibodies (K8010) and Vina Green chromogen (green-colored product, BRR807AS, Biocare Medical). Finally, the nuclei were stained blue with Mayer’s hematoxylin and slides sections were air-dried and mounted with Pertex (Histolab reg.trade mark [cat.nr](http://cat.nr) 00840).

**RESULTS**

Demographic and clinical data for the seven patients can be found in Table 1.

Histopathologic analysis

The biopsy from patient 7 shows a dilated large calibre vessel with thickened fibrotic wall. The lumen is partly occluded by thrombosis, which is undergoing organisation and recanalisation with angiogenesis. The endothelial cells in the vessel stain positively with D2-40 (Figure 1).  The biopsies from cases 5 and 6 each also shows part of a vessel with thickened fibrotic wall, similar to case 7. However, thrombosis was not seen in the lumen of case 5. In case 6, the plane of section did not include the lumen. The biopsies from the other four cases show fibroadipose tissue without evidence of a dilated large calibre vessel with thickened fibrotic wall as is present in the other three cases.

**DISCUSSION**

This represents the first report where an axillary cord is demonstrated conclusively by immunohistochemistry to be caused by a thrombosed lymphatic vessel. The biopsies examined in this study were originally examined by standard cellular pathology at the time they were taken between 1996-98 but results were considered to represent venous thrombosis. The surgeon’s comments at the time were that the cords had the appearance and texture of inflammatory vein/phlebitis, a bit thick and similar to patients’ varices he had operated through the years.

In one of our cases (case 7), we demonstrate that the axillary cord is represented by a dilated lymphatic vessel with a thickened fibrotic wall and occlusion by thrombosis. The lymphatic nature of the vessel is confirmed by immunohistochemical staining with D2-40. Our findings are much in line with previous reports which have indicated thrombotic occlusion of a lymphatic vessel although these other studies did not include confirmation of the lymphatic nature of the vessel by immunohistochemical staining. Thus, it could not be categorically concluded that the vessels were lymphatic vessels (5-7). Our findings are also in keeping with a case report (12) where D2-40 immunohistochemistry confirmed a thickened lymphatic vessel as the cause of the axillary cord. Unusually, in this case the cord developed following a furuncle, rather than the usual sequela of axillary surgery for breast cancer. In this case, however, there is no thrombotic occlusion of the vessel.

Two of our other cases each showed a large vessel identified as lymphatic with thickened walls although thrombotic occlusion was not evident. The sections of the other four cases revealed fibroadipose tissue without a similar large vessel with thickened wall. In our original study from 2001, vessels, identified morphologically at that time as veins, were present in six of the cases (1).  Based on the current re-analysis, the vessels are now regarded as thickened lymphatic vessels. The slides sections from the original study have been discarded. Instead, in the current study, new cut slides sections were examined from the same tissues preserved in the original paraffin blocks. This factor most likely accounts for the apparently discrepant finding of a vessel in only three cases rather than the original six.  It is most probable that the material containing the vessel in the other three cases has been cut out from the tissue paraffin block and is therefore not represented in the newly cut sections.

Understanding the pathogenesis of AWS has eluded investigators. It is a reasonable hypothesis, that the cording represents lymphatic thrombosis because a) the phenomenon occurs after severance of lymphatic vessels and b) lymph does clot (13). Other pathogenic explanations could include a change in the fascia or venous thrombosis, but ultrasound examination failed to identify any structures consistent with either explanation (14).  The ability to snap the cords is consistent with a thrombosed vessel. The triad of Virchow describes the concept of thrombus formation as being the result of one or more of the following broad pathological changes: disruption in flow, endothelial damage and hypercoagulability. Axillary node surgery can clearly account for disruption and stasis in lymphatic flow and damage to lymphatic vessels.

The finding of thrombotic occlusion of the dilated lymphatic vessel with recanalisation of the organized thrombus by angiogenesis corresponds to the transient nature of the axillary cord. The cord resolves as the vessel gradually recanalises. This agrees with the natural history of AWS, showing a reduction of symptoms and normalisation of ROM within 3 months postoperatively (1,15). Thus, the patients with an axillary cord should be reassured and informed that this condition will improve, even without treatment.

**CONCLUSION**

Although only one case this is the first pathological evidence of thrombosis within a confirmed lymphatic vessel from a case of cording. We propose that axillary web syndrome represents lymphatic vessel thrombosis. Recanalisation of the thrombus and its resolution is consistent with the transient nature of the condition.

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**Author Disclosure Statement**

No competing financial interests exist.

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