Validation of a CD30 Enzyme-Linked Immunosorbant Assay for the Rapid Detection of Breast Implant-Associated Anaplastic Large Cell Lymphoma

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Abstract

Background: Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is an uncommon type of non-Hodgkin lymphoma occurring in the fluid or capsule adjacent to textured breast implants. Diagnosis of BIA-ALCL of symptomatic patients requires demonstration of large anaplastic cells with uniform expression of CD30 protein on immunohistochemistry.

Objective: The authors investigated a novel, rapid, office-based, and economic in-situ enzyme-linked immunosorbent assay (ELISA) for screening BIA-ALCL patients.

Methods: A commercially available in-situ ELISA was standardized and validated for patients with confirmed BIA-ALCL diagnosis with clinical isolates. A panel of 9 pathologically confirmed BIA-ALCL patients was screened by serum, plasma, and periprosthetic effusion specimens and compared against serum, plasma, and nonneoplastic delayed seromas in 7 control patients. Statistical analysis demonstrated assay consistency and reliability.

Results: All BIA-ALCL effusions demonstrated CD30 ELISA detection at full and all serial concentrations. BIA-ALCL serum specimens and all control specimens were negative at full concentration and serial dilutions (1:100, 1:250, 1:500, and 1:1000). BIA-ALCL plasma specimens were weakly positive at full concentration and revealed no activity with serial dilution.

Conclusions: This is the first study to demonstrate a viable alternative to CD30 immunohistochemistry for the screening of BIA-ALCL. Our study demonstrates 100% sensitivity in seroma fluid with no detectable CD30 in benign seroma samples. A CD30 ELISA represents a novel, low-cost screening test, which may be used to screen suspicious aspirations of delayed periprosthetic fluid collections in an office-based setting.

Level of Evidence: 3

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Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is an uncommon, distinct form of non-Hodgkin lymphoma.[1,2] The most common presentations of BIA-ALCL are delayed fluid collection (80%) or mass (20%) and may present several years following implantation.[3] Seen in both cosmetic and reconstructive breast cases, there is an estimated risk between 1 in 1000 and 1 in 30,000 women with textured implants.[4] Diagnostic criteria for BIA-ALCL include large anaplastic cells and CD30 immunohistochemistry.[1,2] CD30 is a marker for T and B cell activation and although not exclusively found in BIA-ALCL, it is a useful screening test.

Enzyme-linked immunosorbent assay (ELISA) is frequently used to detect and quantify an antigen in a liquid sample.[5] A primary detection antibody is added to a sample, forming an antigen-antibody complex. The primary detection antibody is either directly labelled with an enzyme (direct ELISA) or is itself attached to a "secondary detection antibody" (indirect ELISA). This method of immune detection is rapid, cost-saving, and characterized by high sensitivity.

Definitive diagnosis of BIA-ALCL requires a several-day waiting period to confirm immunohistochemistry and cytology. Although these steps are necessary for the proper diagnosis, it is much more common to have a benign seroma, and therefore we are interested in developing a quick, cost-effective screening tool to be used with aspiration in the office. Our hypothesis is that an ELISA test for CD30 can rapidly screen for BIA-ALCL.

METHODS

Serum, Plasma, and Seroma Samples

Samples were collected from 16 patients (18 fluid collections) between December 2016 and August 2017. All patients were female and the mean age was 57 years (range, 38-77 years). Nine were confirmed cases of BIA-ALCL based on standard cytology and pathologic criteria. Two of these patients had nonneoplastic effusions on the contralateral breast that were utilized for comparison. Control samples were collected from 7 patients with benign seromas. All specimens were tested at full concentration and serial dilutions (1:50, 1:100, 1:250, 1:500, and 1:1000). This study was conducted with approval of the Institutional Review Board at The University of Texas MD Anderson Cancer Center and informed consent was obtained.

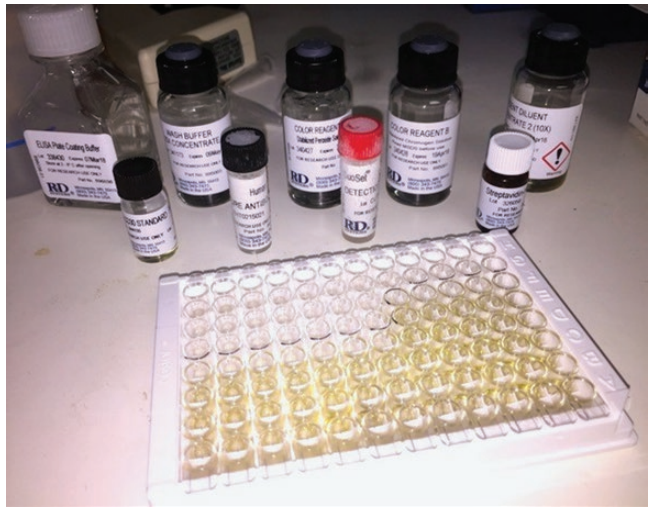

Figure 1. Standard enzyme-linked immunosorbent assay kit demonstrating results from a pathologically confirmed breast implant anaplastic large cell lymphoma patient.

CD30 Enzyme-Linked Immunosorbant Assay

A commercially available ELISA (R&D Systems, Minneapolis, MN) was utilized to measure the concentration of CD30 in effusion, serum, or plasma samples (Figure 1). Briefly, 100 µL of each sample dilution was added to wells and the plate was incubated in the dark at room temperature. Wells were washed, incubated with 100 µL of biotinylated anti-CD30, washed again, and incubated with 100 µL of streptavidin-horseradish peroxidase solution. Finally, 100 µL of stabilized chromogen was added and incubated for 20 minutes and absorbance measured at 450 nm on a photometric plate reader.

Statistical Methods

We employed descriptive statistics to summarize the concentration of CD30 in serial dilutions (1:100, 1:250, 1:500, and 1:1000) and Mann-Whitney-Wilcoxon test to make comparisons. A linear regression model was utilized to determine the threshold of the concentration of CD30 in seroma for BIA-ALCL patients in various dilutions. Sensitivity and specificity were calculated and the model validated. All tests were 2-sided. The analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC) and R (The R Foundation for Statistical Computing).

Table 1. Concentration of CD30 in Seroma Samples

	ALCL positive seroma						Contralateral benign seroma						Benign seroma					
CD30	No.	Mean	Std	Min	Median	Max	No.	Mean	Std	Min	Median	Max	No.	Mean	Std	Min	Median	Max
1:50	9	5507.1	1277.3	3366.1	5873.0	7992.3	2	963.4	190.1	121.9	963.4	1804.9	7	128.7	144.0	3.33	48.3	301.6
1:100	9	4932.6	1369.6	3017.4	4714.9	7918.5	2	881.1	725.7	368.0	881.1	1394.3	7	116.4	129.7	0.74	42.8	274.8
1:250	9	4453.3	1577.1	2868.4	3776.6	7910.3	2	802.2	556.1	409.0	802.2	1195.5	7	119.2	121.2	0.07	72.5	266.1
1:500	9	3907.6	1688.7	2527.9	3351.9	7616.6	2	547.1	353.2	297.4	547.2	796.9	7	133.3	129.7	10.7	84.6	320.6
1:1000	9	3270.1	1761.5	1838.6	2471.5	7183.6	2	239.2	312.0	18.6	239.2	459.8	7	121.1	117.8	32.7	62.1	293.8

Concentration of CD30 (pg/mL) in seroma samples from patients with confirmed diagnosis of breast implant associated anaplastic large cell lymphoma (BIA-ALCL, positive seroma), benign seromas in the contralateral or unaffected breast of those with BIA-ALCL (contralateral bengin seroma), or benign seromas confirmed by cytology (benign seroma). Samples were diluted 1:50, 1:100, 1:250, 1:500 and 1:1000. Neat samples of positive seromas were too strongly positive to register value by CD30 ELISA technique. Max, maximum value; Min, minimum value; Std, standard deviation.

RESULTS

The concentration of CD30 was significantly greater in ALCL seroma in all dilutions (Table 1, $P < 0.001$ for in all dilutions). Overall, there was at least a 10-fold increase in concentration of CD30 detected at all serial dilutions (Figure 2). To overcome the difficulty in diagnosis due to great variation in the CD30 concentration in serial dilutions, we built a practical model to incorporate dilution factors. A seroma sample was considered as BIA-ALCL positive when the concentration of CD30 in this dilution was greater than (2500 − 1.5* dilution) pg/mL (Figure 3). One thousand Bootstrap samples were used to validate the diagnosis model. Both the average sensitivity and specificity remained 100%.

Next, we explored CD30 detection in plasma and serum samples from patients with known BIA-ALCL. Peripheral blood samples were processed to plasma and serum and tested employing the ELISA. Figure 4 shows a minimal detectable CD30 concentration (pg/mL) of representative serum (blue line) and plasma (purple line) samples. Finally, we compared concentrations of CD30 at serial dilutions of seroma fluid samples that included any associated cells (red line) vs samples that were centrifuged to remove the cellular components (yellow line). There are higher detectable CD30 concentrations in samples with cells compared with samples without cells; however, both variations of sampling demonstrate significantly higher CD30 concentrations compared with benign effusions, serum, or plasma.

DISCUSSION

BIA-ALCL represents an uncommon oncologic entity in plastic surgery. It usually presents as a delayed periprosthetic seroma with monoclonal expansion of CD30+ large anaplastic T cells.[6,7] Screening of symptomatic patients requires demonstration of these large cells with uniform membranous expression of CD30 protein on immunohistochemistry.[8,9] Our data demonstrate very low concentrations of CD30 in plasma and serum samples of patients with largely positive CD30 seroma samples and confirmed diagnosis of BIA-ALCL. Such plasma concentrations are consistent with levels of soluble CD30 in healthy controls historically reported elsewhere[10], illustrating the local nature of the disease. This is the first study to report a rapid and cost-effective ELISA to detect the presence of soluble CD30 shed from tumor cells within a fluid sample with excellent sensitivity in BIA-ALCL patients.

Given the heightened concern and awareness of BIA-ALCL combined with the knowledge that complete surgical excision is required for the best clinical management,[2] this is a diagnosis to be made prior to any surgical intervention such as implant exchange or partial capsulectomy; those would not be appropriate management of this disease. This ELISA technique could be adapted similar to rapid in-office or even at-home colorimetric tests such as urine pregnancy tests or glucose dipsticks. The development of this methodology could lead to rapid screening in a setting where there is not enough seroma fluid for cytology and immunohistochemistry, or there is a lower clinical suspicion of BIA-ALCL. Our sample size is small, given the rarity of BIA-ALCL. Although it is considered a tumor marker, CD30 is also expressed normally by activated T and B cells and has been detected in up to 12% of cells in clinically benign lymphocyte-rich seromas.[11] A background of CD30+ T cells is estimated to occur in between 1% and 5% of circulating T cells; a higher concentration occurs in inflammatory and autoimmune conditions.[12,13] Therefore, it is important to note that an ELISA would be employed as a screening method and that definitive BIA-ALCL diagnosis requires further pathology review for cell morphology on cytology and clonal expansion of a T-cell population.

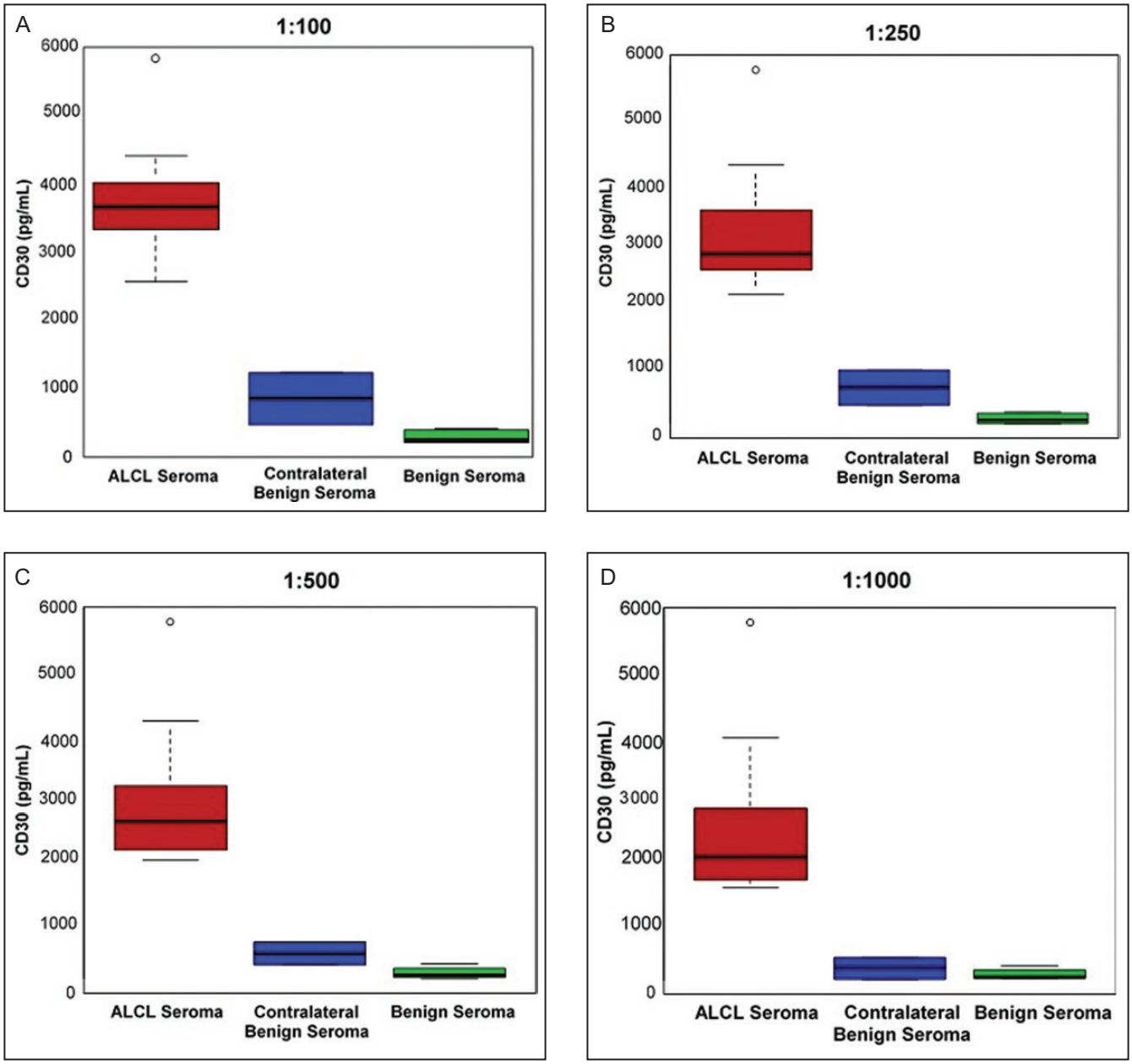

Figure 2. Concentration of CD30 (pg/dL) in seroma samples from patients with confirmed diagnosis of breast implant-associated anaplastic large cell lymphoma (ALCL; positive seroma), benign seromas in the contralateral or unaffected breast of those with breast implant-associated anaplastic large cell lymphoma (contralateral benign seroma), or benign seromas confirmed by cytology (benign seroma). Samples were diluted 1:50 (not shown), (A) 1:100, (B) 1:250, (C) 1:500, and (D) 1:1000.

CONCLUSIONS

The development of a rapid screening test based on ELISA and immunohistochemistry would easily facilitate the screening process in patients with high clinical suspicion of BIA-ALCL as well as referral to a cancer center for additional work-up and treatment. Larger scale, prospective studies are warranted.

Disclosures

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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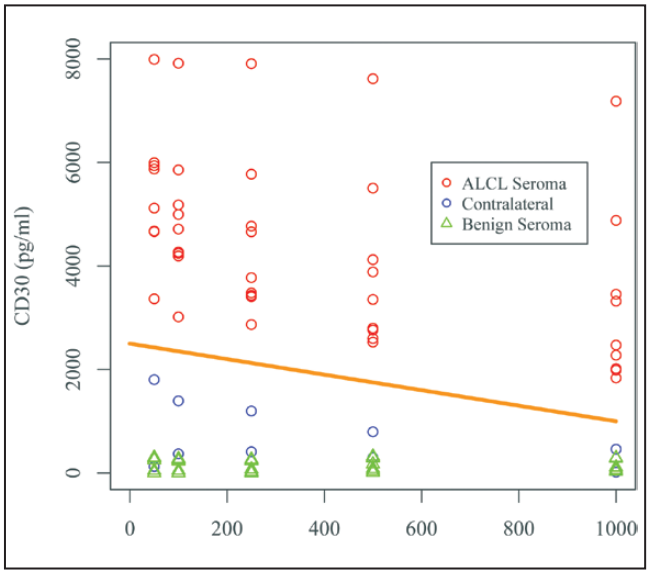

Figure 3. The concentration of CD30 varies from 18.6 pg/mL to 1804.9 pg/mL in controls and from 1838.6 pg/mL to 7792.3 pg/mL in anaplastic large cell lymphoma (ACLC) seroma for different dilutions. To overcome the difficulty in diagnosis due to great variation in the concentration of CD30 in serial dilutions, we built a practical model to incorporate dilution factors. A seroma sample was considered as anaplastic large cell lymphoma positive when the concentration of CD30 in this dilution was greater than (2500 − 1.5* dilution times) pg/mL. The sensitivity and specificity are both 100% by using this model. Figure 3 shows the diagnosis model to separate anaplastic large cell lymphoma positive and negative samples. One thousand Bootstrap samples were used to validate the diagnosis model. Both the average sensitivity and specificity remained 100%.

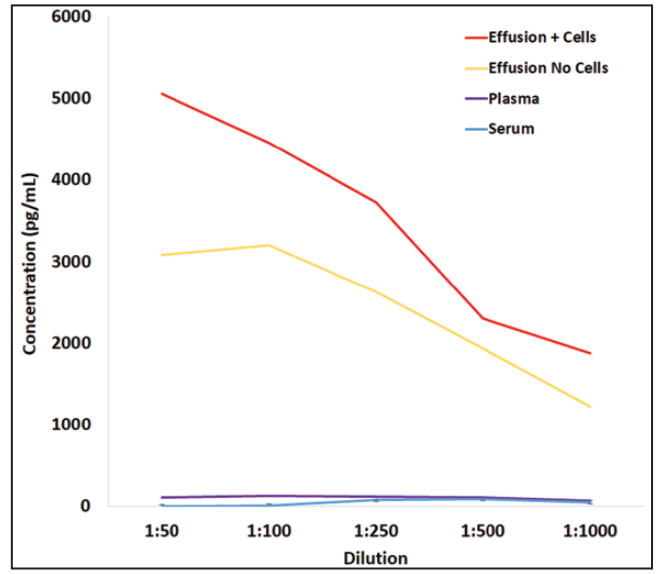

Figure 4. Concentration of CD30 (pg/dL) in seroma samples with cells (red line) or without cells (yellow line), serum (blue line), or plasma (purple line) from patients with confirmed diagnosis of breast implant associated anaplastic large cell lymphoma. Samples were diluted 1:50, 1:100, 1:250, 1:500, and 1:1000.

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