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Real-time ultrasound elastography for assessment of response to brentuximab vedotin treatment in relapsed and refractory Hodgkin lymphoma

E. SQUILLACI, M. ANTONICOLI, G. MANENTI, F. BOLACCHI

Department of Diagnostic and Molecular Imaging, Radiation Therapy and Interventional Radiology, University Hospital Tor Vergata, Rome, Italy

Abstract. – OBJECTIVE: To evaluate the application of ultrasound elastography (ES) in monitoring treatment response to brentuximab vedotin (Seattle Genetics, Seattle, WA, USA).

PATIENTS AND METHODS: Patients were selected when suffering from relapsed and refractory Hodgkin Lymphoma (HL). Our research investigated if the interim of ultrasound ES is a predictive value for treatment outcome in patients treated with brentuximab vedotin.

RESULTS: 30 patients with refractory HL were enrolled. After treatment with brentuximab vedotin, 14 patients were classified as responders and 16 were classified as non-responders. At baseline, there was no difference between the groups both in the strain ratio ($z = 1.1$, $p = 0.3$) and in the volume ($z = -0.3$, $p = 0.8$). While after treatment there was a difference between the groups both in the strain ratio ($z = -2.09$, $p < 0.05$) and in the volume ($z = 4.1$, $p < 0.001$).

CONCLUSIONS: Real-time elastosonography could be a reliable tool for the assessment of refractory Hodgkin lymphoma response to brentuximab vedotin treatment and help to identify patient with improved clinical outcome early during treatment. Results indicate that changes in ultrasound elastosonography parameters are correlated with the clinical and pathologic response of patients. These findings could pave the way for establishing protocols for the clinical applications of ultrasound elastography techniques in therapy response monitoring.

Key Words:
Ultrasound elastography, Hodgkin lymphoma, Brentuximab vedotin treatment.

Introduction

Lymphomas are a heterogeneous group of lymphoproliferative malignancies with differing patterns of behavior and responses to treatment. They represent approximately 4% of newly diagnosed cancers each year and are more common in developed countries[1].

Recent studies indicate an incidence rate, per 100,000 persons per year, of 33.6 for all lymphoid neoplasms, with a rate of 26.1 for B-cell neoplasms and 2.6 for Hodgkin Lymphoma (HL). For these patients, chemotherapy is the mainstay of the treatment. Early prediction of response could improve the personalization of the treatment by selecting only those patients who are most likely to benefit from it, and by intensifying the treatment in well-responding patients.

Fluorine 18 fluorodeoxyglucose (FDG) positron emission tomography (PET) integrated with computed tomography (CT) has emerged as a powerful imaging tool in cancer staging and in predicting cancer's response to therapy[2-6].

Particularly, PET-CT imaging has a well-established role in the assessment of treatment response in patients with HL. In this light, an interim 18F-FDG-PET-CT after only two cycles of chemotherapy has been shown to be a sensitive prognostic indicator of treatment outcome in patients with advanced stage HL[7].

Elastosonography (ES) is a recently developed dynamic imaging technique which provides information about the relative stiffness of the tissues by measuring the degree of strain-related distortion under the application of an external force[8,9].

In this context, elastography consists in applying a quasi-static pressure on the examined tissue surface and in estimating the induced strain distribution by tracking the tissue movement. On the other side, raw ultrasound data consist of radiofrequency data which are acquired continuously, and the elastograms are generated by estimating the strain between the sequential frames[10,11].

Corresponding Author: Francesca Bolacchi, Ph.D; e-mail: francesca.bolacchi@libero.it

ES inherits the advantages of ultrasound imaging such as low cost, rapid imaging speed, portability and lack of ionizing radiation use. Furthermore, no contrast agent injection is needed in this modality. This is because of the fact that the differentiable elasticity and stiffness, as main sources of image contrast, are caused by changes in the physical properties of the examined tissue. Over the last five years, this method has been mainly applied for the study of solid lesions of the breast, prostate, liver and thyroid. ES with its various technical implementations seems to be also a promising tool that complements standard ultrasonography for the diagnosis of malignant superficial lymph nodes[11-13].

It has been shown that chemotherapy might decrease cancer-associated fibroblasts content inducing a marked alteration in cancer stroma that results in tumor softening[14]. Similarly to metabolic changes, modifications of tumor stiffness might occur earlier than dimensional ones. ES has shown promising results as an early predictor of tumor therapy response in breast cancer patients who undergo neoadjuvant chemotherapy[15].

This study investigated for the first time, the application of ES in monitoring chemotherapy response in patients with HL heavily pretreated patients with relapsed and refractory HL treated with brentuximab vedotin (Seattle Genetics, Seattle, WA, USA).

Patients and Methods

Study Protocol

The study included 30 patients (16 males and 14 females; mean age 38 ± 10 years) with histologically confirmed CD30-positive classical HL that was refractory to or relapsed after standard chemotherapy. Other inclusion criteria were: fluorodeoxyglucose-avid disease by PET and measurable disease of at least 1.5 cm in diameter by CT and localized to superficially located lymphatic stations, namely laterocervical, supraclavicular, axillary or inguinal lymph nodes.

Patients were excluded if they had not superficial lymphatic stations affected by the disease.

Before the treatment administration, all patients underwent to 18F-FDG PET-CT total body scan for staging purpose. Given that a biopsy was required to obtain the histological diagnosis, only patients with involved lymph nodes other than biopsied ones were included. ES was performed at the level of hypermetabolic lymph nodes that were at least 5 cm distant from the biopsy site. If more than one lymph node was involved, all evaluable lymphadenopathies were checked.

Ultrasound B-mode and elastography and volume data were acquired from the hypermetabolic lymph nodes, at the following times: before chemotherapy, and about the third cycle of administration of the drug. Here we report the result of a single-center assessment of the role of ES imaging during therapy with brentuximab vedotin in patients with relapsed and refractory HL. We investigated whether interim ES sustain its predictive value for treatment outcome in patients treated with brentuximab vedotin. The experimental protocol was approved by the Ethics Committee of our institution and written informed consent was obtained from all volunteers in accordance to the Declaration of Helsinki.

Study Treatment

Brentuximab vedotin was administered i.v. to all thirty patients on day 1 of each 21-day cycle with a dose of 1.8 mg⁄kg and within 120 (± 15) min. The number of chemotherapy cycles varied in a range of 8-16, evaluating periodic clinical-labs data in order to exclude the possible progression of the disease or an excessive toxicity of the treatment.

If the latter situation occurred, the entity of toxicity was assessed and according to its severity the dose was reduced within 1/3 of the initial dose; if a further dose reduction was needed the study treatment was interrupted.

Equipment and Ultrasound and Elastogram Techniques

The ultrasound scan and RTE were performed using the same ultrasound system (IU22, Philips Medical Electronics Systems N.V. Corporation, Eindhoven, The Netherlands) and the ultrasound probe (7.5 MHz) and volumetric linear probe V-16 (5-12 MHz). Philips ultrasound system (iU22) using a multifrequency linear probe (12-5 MHz). All the ultrasound data in this study were collected by the same physician following standardized protocols for data acquisition.

Each lymph node was studied with elastosonography an assessment of the volume.

Use of the iU22 Philips system and probe provided repeatably and not highly operator-dependent measurements, since on this system, the stress for the strain elastography is provided by spontaneous body movements (mainly cardiac),

thus, the operator simply has to hold the probe gently in contact with the gel on the skin, even if the movement is not provided by the scanner.

The system allows to verify in real-time the appropriateness of tissue deformation in order to obtain a good-quality elastogram.

The box for ES analysis was selected to include the enlarged lymph node and part of the surrounding structures mainly located near the sternocleidomastoid muscles, even if axillary and inguinal nodes are also investigated. For each studied lymph node, the transducer focus was set at a tumor center depth and kept consistent throughout the study. The resulting elastography images were displayed using a 256-color map of strain using a scale from red (highest strain, soft) to blue (low strain, hard).

Mean elasticity (RTE-Me) values were calculated. RTE-Me was described in arbitrary units [a.u.]. The elastographic images with the concomitant histogram were obtained positioning two Region of Interest (ROI) on the lymph node in the longitudinal plane (ROI-1^{node}) and in the majority of the cases on the sternocleidomastoid muscle in the longitudinal plane (ROI-2^{muscle}). An indicator in the screen provided the user with real-time feedback on the appropriate amount of deformation for the elastogram. The total amount of deformation used to compute the strain elastogram is the sum of inherent, or physiologic, patient motion plus the external compression of the transducer. It displayed instantaneous or momentary tissue. The elastosonogram analysis began when the strain indicator of the probe expressed the correct pressure of the operator. The colors in the ROI varied from blue to red to show the relative hardness and softness of areas inside the ROI. The probe was kept in position manually while the patient was in the rest position. The entire examination lasted approximately 14 ± 2.8 minutes for each patient. The color-coded images were analyzed on a personal computer using the QLAB (developed by Philips Medical Systems), which permitted us to selected a 30 frame images/cine-loop. The references (ROI-1^{node}) and (ROI-2^{muscle}) measuring the strain index (SI=ROI1/ROI 2). For quantification, all pixel data in the colored image were transformed into a histogram and RTE-Mean elasticity (RTE-Me) values were calculated. RTE-Me was described in arbitrary units [a.u.] (Figure 1).

The study of the volume of the lymph node pathological localized within latero-cervical was performed with conventional ultrasound technique (Figure 2).

It was used a probe volume superficial V-16 Philips and Philips iU22 system (Philips Medical Electronics Systems N.V. Corporation, Eindhoven, The Netherlands).

The measurements are carried out automatically by the system, repeatable, which allows you to calculate the volume of the lesion. In addition a qualitative evaluation was performed based on a color-based assessment.

Tumors showing any appreciable softening on interim ES images, visualized as color change from relatively to baseline ES, were classified as "responders", while those not showing significant stiffness modification or, contrarily showing an appreciable hardening, were classified as "no responders". To this purpose, a concordant opinion was required among two different physicians. In case of disagreement, a third opinion was asked.

Data PET/CT

The PET/CT system Discovery ST16 (GE Medical Systems, Knoxville, TN, USA) was used to assess ^{18}F-FDG distribution in all patients by a 3-D mode standard technique in a 128x128 matrix. The system combined a high-speed ultra 16-detector row (912 detectors per row) CT unit and a PET scanner with 10,080 bismuth germanate crystals in 24 rings. All patients fasted for at least 5 hours before ^{18}F-FDG i.v. injection; serum glucose level was ‹110 mg/dl in all of them. All subjects were injected with 2.5 MBq/kg ± 10% (210-410 MBq) of ^{18}F-FDG i.v. and hydrated with 500 ml of i.v. saline sodium chloride (NaCl) 0.9%. Whole body PET/ceCT (contrast-enhanced CT) scan was performed approximately 60 min after ^{18}F-FDG injection with the following parameters: 120-140 kV, automatic milliamperage (limit 330-350 mA), thickness 3.750 mm reconstructed at 1.25 mm, acquisition mode 27.50/1.375:1, gantry rotation time 0.6 s, large FOV, matrix 512 × 512; concerning the nonionic iodinated contrast material (370 mg/ml, 420 mgI/kg) a volume of 100-120 ml was administered at 3 ml/s. The first scan included the upper abdomen with a 30-s delay from the injection start: the second one was extended from the neck to the pelvis with a 60-s delay. Finally, with a delay of about 3 min after the i.v. contrast injection, a brain CT was performed.

Treatment Response Evaluation

Response assessment was evaluated according to the Revised Response Criteria for Malignant

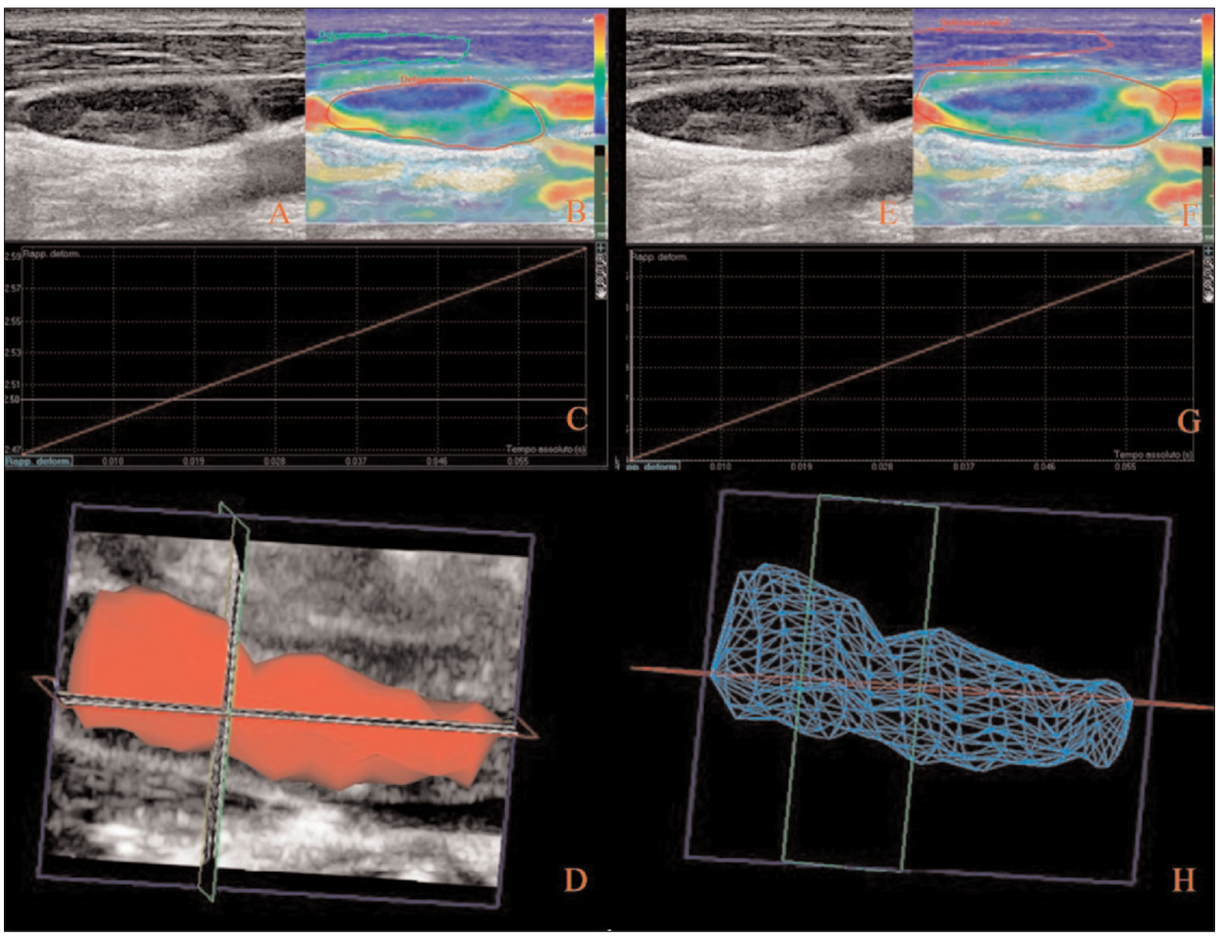

Figure 1. 39-year-old male with worse clinical outcome images shows the analysis conducted on laterocervical pathological lymph node at T0 ***(A,B,C,D)*** before the start of chemotherapy and at time T1 ***(E,F,G,H)*** after administration of the third cycle of chemotherapy. ***(A,E)*** conventional axial B-mode ultrasound scan and superimposed, ***(B,F)*** sonoelastogram was obtained by positioning two region of Interest (ROI) on the lymph node in longitudinal plane (ROI-1^{node} circular line in red) and on the sternocleidomostoid muscle in longitudinal plane (ROI-2^{muscle} circular line in green). ***(C,G)*** The graph shows strain index (SI = ROI1/ROI 2) calculated between the laterocervical pathological lymph node and the sternocleidomastoid muscle ***(D,H)*** three-dimensional volumetric analysis of lymph node. (Scale unit: 5 mm).

Lymphoma[16] performing a CT scan; subsequently, patients were classified as having a response or progressive disease according to the result of the CT examination.

Statistical Analysis

Clinical data were collected at baseline and after 3 cycles of therapy. Continuous variables (volume and strain ratio) were compared between responders and non-responders at baseline and after treatment using the Mann-Whitney test. The level of statistical significance was set at $p < 0.05$. Statistical analysis were performed using STATA MP11 (StataCorp LP, College Station, TX, USA).

Results

We enrolled 30 patients with refractory HL. Table I summarizes the clinical characteristics of the enrolled patients before the initiation of brentuximab treatment.

After treatment, 14 patients were classified as responders and 16 were classified as non-responders (Table II).

At baseline, there was no difference between the groups both in the strain ratio ($z = 1.1$, $p = 0.3$) and in the volume ($z = -0.3$, $p = 0.8$).

While after treatment there was difference between the groups both in the strain ratio ($z = -2.09$, $p < 0.05$) and in the volume ($z = 4.1$, $p < 0.001$).

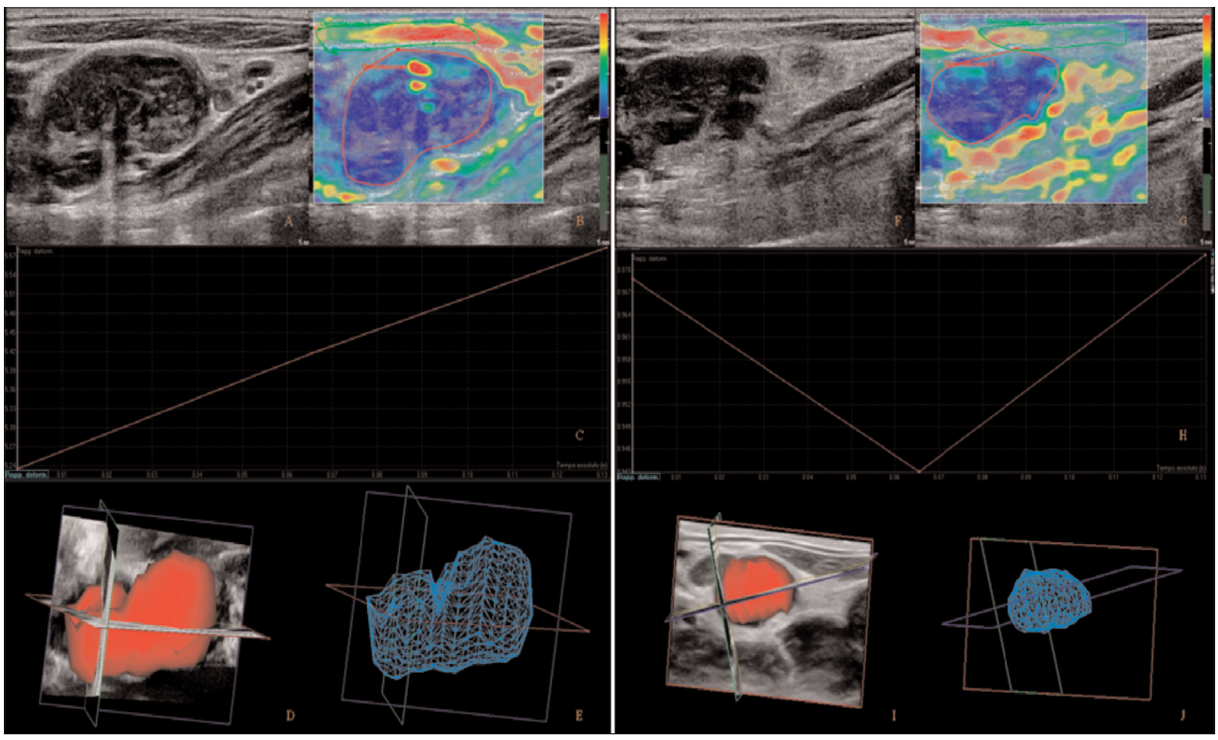

Figure 2. 29-year-old male with good response to therapy; images shows the analysis conducted on laterocervical pathological lymph node at T0 ***(A,B,C,D,E)*** before the start of chemotherapy and at time T1 ***(F,G,H,I,J)*** after administration of the third cycle of chemotherapy. ***(A,F)*** conventional axial B-mode ultrasound scan and superimposed, ***(B,G)*** sonoelastogram was obtained by positioning two region of Interest (ROI) on the lymph node in longitudinal plane (ROI-1^{node} circular line in red) and on the sternocleidomastoid muscle in longitudinal plane (ROI-2^{muscle} circular line in green). ***(C,G)*** The graph shows strain index (SI = ROI1/ROI 2) calculated between the laterocervical pathological lymph node and the sternocleidomastoid muscle; three-dimensional volumetric analysis of lymph node at T0 ***(D, E)*** and at T1 (I, J). (Scale unit: 5 mm).

Table I. Clinical characteristics of the enrolled patients before the initiation of brentuximab treatment.

Male, n (%)	16 (54)
Female, n (%)	14 (46)
Mean age, years (± DS)	38 (± 10)
Lymph node strain ratio, median (range)	0.41 (0.11-0.81)
Lymph node score, median (range)	2 (1-4)
Lymph node volume, median (range)	5 (3-8)

Discussion

Ultrasonography represents the recommended imaging method for the evaluation of superficial lymph nodes thanks to the advantages of high resolution, low costs and the possibility of a real-time examination[17].

The widely accepted morphologic US criteria to identify a malignant lymph node include echogenicity, maximum short diameter, long to

Table II. Clinical characteristics of the enrolled patients after 3 cycles of brentuximab treatment.

	Responders (n = 14)	Non-responders (n = 16)
Male, n (%)		
Female, n (%)		
Mean age, years (± DS)		
Lymph node strain ratio, median (range)	0.49 (0.30-0.60)	0.65 (0.40-0.90)
Lymph node score, median (range)	2 (1-3)	3 (2-4)
Lymph node volume, median (range)	5 (3-7)	2 (1-4)

short axis ratio, absence of hilus, presence of necrosis and fusion tendency. The further evaluation with Doppler and color Doppler systems improves the identification accuracy of malignant node[18]. The introduction of advanced US techniques such as contrast-enhanced ultrasound (CEUS) and elastosonography (SE) potentially allow to increase the accuracy of the differential diagnosis between benign and malignant lymph nodes[19].

In 1991, Ophir et al[20] described this possibility by measuring the tissue compliance, considering the elasticity which represents an intrinsic tissue characteristic able to prevent the tissue displacement when it is under pressure. An elastogram is obtained by displaying the results of tissue compression on a monitor after applying a mechanical force on a soft tissue such as the lesion and the area around it. Pathological disorders can cause alteration of the elasticity of the tissue, a feature that varies depending on the different type of tissues and their structure. SE has been applied for the examination of different organs such as breast, liver, prostate, skin and rectum in order to better understand morphologic findings and in some cases to predict the response to treatment. Research[21] showed that tumor stiffness is associated with tumor progression and recurrence. Hodgkin lymphoma (HL) is a lymphoid malignancy of B-cell origin which is classified, according to 2008 WHO classification, into either nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) or classical Hodgkin lymphoma (CHL)[2]. Nowadays, thanks to the improvements in detection, management and treatment of the disease, it represents a highly curable malignancy with 85% of patients affected by Hodgkin lymphoma, that become long-term survivors[22]. HL is refractory or relapse after first line therapy in a percentage of 20-30% of patients; these patients undergo a second line or a salvage chemotherapy regimen usually followed by a stem cell transplant (STC) as consolidation. Nevertheless, the 50% of cases do not respond or present disease recurrence with a poor prognosis[23]. In these considerable group of patients, several studies have documented the efficacy of Brentuximab Vedotin in terms of median progression-free survival. As an antibody drug conjugate (ADC) targeting CD30 receptors, Brentuximab Vedotin (ADCETRIS-Seattle Genetics, Seattle, WA, USA) is internalized by Reed-Sternberg and most of the other HL cells expressing the receptor. Subsequently, the microtubule-disrupting agent monomethyl auristatin E (MMAE) is released within the cytoplasm inducing cell death and apoptosis[24]. Among the 400-450 lymph nodes of the human body 60-70 are located in the neck region and these are more frequently involved in HL than other superficial locations. The evaluation of superficial lymph nodes can be performed using an elasticity score that takes in consideration the appearance of the node or by measuring its relative stiffness or strain ratio[25]. In this light, we investigated cervical lymph nodes with RTE selecting them on the basis of PET/CT involvement (SUV Max) and avoiding nodes with large necrotic areas or fused lymph node packages. In literature, data related to the node involvement in lymphomas and strain are very limited. The results from studies employing SE in the differential diagnosis of superficial lymph nodes are controversial. Indeed, in most cases quantitative strain ratio failed in differentiating nodes affected by lymphomas node from other nodal malignancies or even benign diseases such as tuberculosis (TBC)[26]. In consideration of the results of our study, we stated that although strain ratio could result non-conclusive in evaluating node involvement in HL, this could be used as a follow-up marker together with the distribution and amount of the hard areas (blue) described by our qualitative scoring system. We analyzed strain data at T0 and T1 where then compared with usual morphological and 3D volume data obtained with our 3D linear transducer.

Conclusions

We found out that certain elastographic patterns are predictive of Brentuximab therapy response and early response to treatment could also be identified on the basis of the strain ratio reduction after 3 drug cycles; instead, volume analysis and usual morphological changes of the node structure failed to detect the early response. Our study shows, however, some limitations. Firstly, the restricted selection criteria and the prospectively gated data reduced the number of the patients enrolled in our study. Secondly, we performed the US scan only on the cervical node with the highest SUV Max at PET/CT examination before treatment. Currently, the main role of US elastosonography is to increase the accuracy in differential diagnosis between benign and malignant lesions and to identify eventual lymph nodes metastasis. A limited number of studies

recognized the utility of this technique in the prediction of response to neo-adjuvant chemotherapy and in the follow-up of treatment response in solid tumors. To the best of our knowledge, this is the first study concerning drug response prediction and early response detection in lymph nodes. In conclusion, we suggest that lymph nodes stiffness could be useful in monitoring drug response in refractory HL. Detection of the patients with early response could provide important information to the clinician eventually affecting, in the future, the management of therapy, as the number of cycles administered and the overall period of treatment. On the basis of this insight, further research is needed to validate US sonoelastography in monitoring systemic drug treatment response in HL.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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