

## THE ROLE OF LYMPHOSCINTIGRAPHY IN THE DIAGNOSIS OF LYMPHEDEMA IN TURNER SYNDROME

C. Bellini, E. Di Battista, F. Boccardo, C. Campisi, G. Villa, G. Taddei, C. Traggiai, A. Amisamo, P. Polo Perucchin, C.S. Benfenati, E. Bonioli, R. Lorini

Neuromuscular Diseases Unit (CB), Service of Nuclear Medicine (GV,GT), Endocrinology Unit (CT,AA,PPP,CSB,RL), Medical Genetics Unit (EB), Department of Pediatrics, University of Genoa, G. Gaslini Institute and Section of Lymphatic Surgery and Microsurgery (FB,CC), Department of Surgery, S. Martino Hospital, University of Genoa, Genoa, Italy

### ABSTRACT

*Lymphedema can be present in patients affected by Turner syndrome (TS) with the dorsum of the hands and feet most commonly affected. This lymphedema results from underdevelopment of the lymphatic system before birth, and it usually decreases during childhood. The aim of our study was to evaluate the role of lymphoscintigraphy as a diagnostic tool in patients with TS to assess possible impairments in the lymphatic system. Eighteen patients with TS were karyotyped to confirm diagnosis and were evaluated by lymphoscintigraphy. Lymphatic dysfunction was demonstrated in 15/18 patients. Lymphoscintigraphic studies showed: 1) lymphatic channels, 2) collateral lymphatic channels, 3) interrupted lymphatic structures, and 4) lymph nodes of the deep lymphatic system. Our data demonstrate that lymphoscintigraphy should be mandatory not only in patients affected by Turner syndrome with signs of lymphatic dysplasia but also in those with minimal or absent signs of lymphatic impairment in order to obtain a very early diagnosis and to provide substantial information for possible medical or surgical treatment.*

**Keywords:** Turner syndrome, lymphoscintigraphy, congenital lymphatic dysplasia, lymphedema

Turner syndrome occurs in 1 out of every 2,500 to 3,000 live female births. The syndrome is caused by the absence of the second sex chromosome (partial or complete, homogeneous or mosaic) in a phenotypic female. The physical phenotype of TS individuals may vary greatly. Although there is no cure, some treatments for the symptoms are available. Lymphedema is present in about 70% of patients with Turner syndrome with the hands and feet most commonly affected. The lymphedema may appear at any age, and it is the result of primary lymphatic dysplasia that interferes with the function of the lymphatic system. Manifestations and management of TS have recently been reviewed extensively (1-4).

Lymphedema in utero is a major feature of TS. It is well known that a typical diagnostic clue to TS in the neonatal period is swelling of the hands and feet, which, together with webbing of the neck and a distinctive shape of the ears, points to lymphatic system maldevelopment. The occurrence of lymphatic impairment was demonstrated in TS (5-7), while a possible correlation between lymphedema and cardiac malformation is still under debate (1,6).

Lymphoscintigraphy offers objective evidence to help distinguish lymphatic pathology from non-lymphatic causes of edema. It highlights the accumulation of

lymphatic fluid in the interstitium as the cause of swelling, which is most evident in the limbs (8). We recently demonstrated that lymphoscintigraphy should be carried out on all patients with signs of lymphatic dysplasia, including those with minimal and initial signs of lymphatic impairment, in order to obtain a very early diagnosis (9).

The aim of this study was to evaluate the role of lymphoscintigraphy as a diagnostic tool in TS patients in order to have an early assessment of the possible impairment of the lymphatic system.

## *PATIENTS AND METHODS*

### *Patients*

Individuals with TS were recruited for lymphoscintigraphic study from among the patients referred to the Auxo-Endocrinological TS Center of our Department.

Lymphedema was staged on the basis of the Consensus Document of the International Society of Lymphology (10). Stage 0 refers to a latent or sub-clinical condition where swelling is not evident despite impaired lymph transport. Stage I represents an early accumulation of fluid that is relatively high in protein content as compared with "venous" edema and subsides by elevating the limb and pitting may occur. In Stage II, limb elevation alone rarely reduces tissue swelling, and pitting is manifest. Stage III encompasses lymphostatic elephantiasis where pitting is absent and trophic skin changes such as acanthosis, fat deposits, and warty overgrowths develop. Within each Stage, severity based on limb volume difference can be assessed as minimal (<20% increase), moderate (20-40% increase), or severe (>40% increase). These Stages only refer to the physical condition of the extremities.

All patients or parents received appropriate information and gave written informed consent approving the procedure.

### *Lymphoscintigraphy Methods*

Lymphoscintigraphy was performed using a modified version of a previously described protocol (11).

### *Radiotracer*

We used microcolloidal sulfide particles labeled with Technetium-99m (Tc-99m) (Nanocoll®; GE Healthcare, UK), 5MBq (0.135 mCi) in 0.3 ml each, with a particle diameter ranging from 20 to 80 nm. To evaluate the deep lymphatic circulation, two aliquots of tracer (0.1 ml each) were injected into the II and III metacarpal and metatarsal-phalangeal spaces of the hands and feet while two shares of tracer were administered intradermally in the second and third interdigital spaces of the hands and feet in order to evaluate the superficial lymphatic circulation. All injections were carried out simultaneously in younger patients, while we preferred to inject lower and upper limbs at different times in patients above 4 years of age. Subfascial injection of radiotracers should be used to investigate the deep lymphatic system of the limbs, followed by the epifascial and suprafascial route of administration in order to obtain better differentiation of the various kinds of edema.

### *Parameters for obtaining images*

Lymphoscintigraphy for lymphatic mapping involves dynamic imaging (flow imaging) and static (late) imaging. The dynamic study is performed shortly after the injection of the radiocolloid (which is why the injection of the colloid should be performed on the gamma camera table) and at times can visualize the afferent lymphatic vessels leading to the regional basin. A gamma camera with a large field of view and a low energy, high resolution, parallel-hole collimator was used (GE Millennium, USA). We also performed a dynamic study to obtain a number of serial images over a short duration. Our approach involves the dynamic acquisition of 60 frames of 20 seconds in a

**TABLE**  
**Lymphoscintigraphic Study Results in 18 Patients with Turner Syndrome**

	Chromosomal abnormality	LE* staging at birth	Age at procedure	Neck webbing	Lymphoscintigraphic pattern	T1	LE staging at lymphoscintigraphy	Lymphatic System Impairment
1	45,X0 (74%)/ 47,XXX (24%)/ 46,XX (2%)	0	2.8	+	Delay right leg	13	0	Lymphatic hypoplasia
2	45,X0	II (feet & legs) I (Hands)	3.5	++	Delay, interrupted lymphatic structures, reduced tracer uptake by inguinal lymph nodes, back-flow right arm	28	II	Mixed lymphatic impairment (various degree of lymphatic aplasia, hypoplasia, and hyperplasia)
3	45,X0 (88%)/ 46,XX (12%)	0	3.8	++	Delay, interrupted lymphatic structures	22	0	Lymphatic aplasia with various degrees of hypoplasia
4	45,X0 (84%)/ 47,XXX (16%)	0	6.8	+	Normal	<10	0	Normal
5	45,X0	0	12.2	+	Normal	<10	0	Normal
6	45,X0 (80%)/ 46,X,r(X) (20%)	I (feet)	12.3	+	Interrupted lymphatic structures (Deep circulation) right arm and left leg. Compensatory superficial circulation	26	0	Lymphatic aplasia
7	45, X0 (89%)/ 46,X,del(X)(q25 qter) (11%)	0	12.5	++	Interrupted lymphatic structures (Deep circulation) Absence of visualization of right inguinal lymph nodes	27	0	Lymphatic aplasia
8	46,XX/45,X0 (84%)	0	12.5	+	Interrupted lymphatic structures (right leg)	18	0	Lymphatic aplasia
9	45,X0	0	14.3	++	Delay both arms	13	0	Lymphatic hypoplasia
10	45,X0 (80%)/46,XX	0	15.5	++	Slight delay right arm (Superficial circulation)	12	0	Lymphatic hypoplasia
11	45,X0	0	15.8	+	Delay deep circulation left leg and left arm, and delay superficial circulation right leg	13	0	Lymphatic hypoplasia
12	45,X0	I (hands & feet)	18.6	+++	Shunt deep vs superficial circulation (both arms and legs. Delay superficial circulation both legs. Interrupted deep lymphatic structures more evident right leg	22	II	Lymphatic aplasia with various degrees of hypoplasia
13	45,X0	0	22.0	++	Delay both legs	15	0	Lymphatic hypoplasia
14	45,X0	0	23.3	++	Delay left arm (Deep circulation)	12	0	Lymphatic hypoplasia
15	45,X0	0	24.2	+	Shunt deep vs superficial circulation with slight back-flow (left leg)	18	0	Lymphatic hypoplasia (deep circulation) Possible lymphatic hyperplasia
16	45,X0	I (hands & feet)	28.7	++	Back-flow right leg	15	0	Lymphatic hyperplasia
17	45,X0	0	29.8	+	Shunt deep vs superficial circulation (both legs)	13	0	Lymphatic hypoplasia
18	45,X0	0	38.4	+	Normal	<10	0	Normal

\*LE=lymphedema

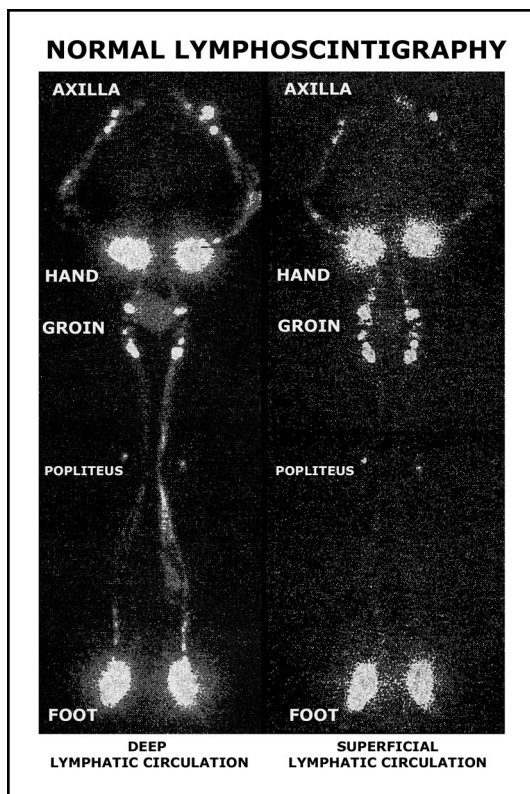


Fig 1. Normal lymphoscintigraphic patterns. Panels A1 and A2. Case 4. Normal deep (A1) and normal superficial lymphatic (A2) circulation. Anatomic landmarks are labeled.

matrix of 128 x 128 begun immediately after the injection of the tracer. The gamma-camera is positioned automatically as closely as possible and in front of the patient, who is in a supine position. At the end of the dynamic acquisition, the study continues with static images using a matrix of 56 x 256; acquisition time was 600-900 seconds.

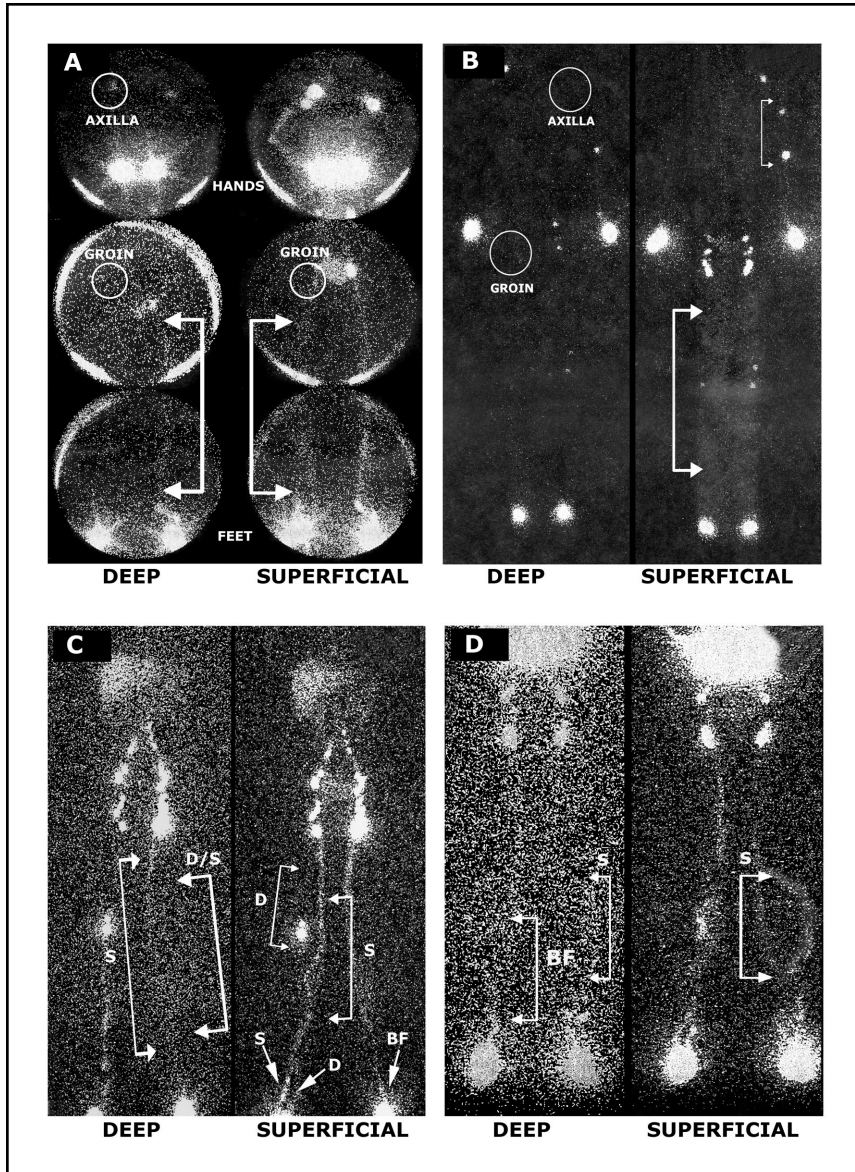
Clearance rate and regional lymph node measurements were performed. Semi-quantitative evaluation of lymphatic drainage was established by a numeric index (transport index: TI) calculated by the formula  $TI = K + D + 0.04T + N + V$ , where K stands for lymphatic transport kinetics (scores for no delay, low grade delay, extreme delay, and lack of transport are 0, 3, 5, and 9,

respectively), D is the distribution pattern of the tracer (scores for normal, partial diffuse, diffuse, and transport stop are 0, 3, 5, and 9, respectively), T stands for how long it takes (in minutes) for the tracer to appear before it can be observed in the lymph nodes (no appearance in 9) and scans are carried out until these data have been obtained, though however, for no longer than one hour, N is the duration (in minutes) that the tracer appears and can be observed in the lymph nodes (no appearance is 9), V is the ratio between the visualization of lymph vessels and graft (nodes and vessels, respectively, clearly demonstrated, faint visualization, hardly recognizable, or no visualization are 0, 3, 5, and 9, respectively). Each parameter ranges from 0 to 9, so total evaluation ranges from 0 (normal) to 45 (pathological). Transport index in healthy extremities was less than 10 (11,12).

Sedation is not usually required for a technically satisfactory examination but in some patients who cannot cooperate, mild sedation may be necessary. In our study, following injection into the lower extremities, a massage was carried out and lukewarm packages were positioned on the inoculation sites. The younger patients (less than 4 years of age) were then allowed to carry out spontaneous motor activity for 10 minutes. Lastly, if necessary, they were restrained so as to optimize images. Older children and adults were allowed to have 5 minutes of spontaneous motor activity after injection.

## RESULTS

Thirty-two patients were selected for the study. Six refused the lymphoscintigraphic procedure. Eight patients presented partial or complete X-monosomy affecting <70% of cells and were excluded. Eighteen patients were eventually enrolled into the study. No adverse effects of the procedure were observed. Data concerning the patient's lymphedema staging, transport index, and results are summarized in the *Table*.



**Fig. 2. Lymphoscintigraphic impairment in Turner syndrome. Deep and superficial lymphatic circulation study.** Panel A. Case 2. Mixed lymphatic impairment. Deep: Severe bilateral hypoplasia of limb circulation (arrows); nearly absent tracer uptake by right inguinal lymph nodes (empty circle; groin); hypoplasia of bilateral arm circulation and absent tracer uptake by right axillary lymph nodes (empty circle; axilla). Superficial: Hypoplasia of right limb circulation (arrows); absent tracer uptake by inguinal lymph nodes (empty circle; groin); normal left limb superficial circulation. Panel B. Case 6. Deep: Bilateral hypoplasia of right limb circulation and normal left limb circulation; absent tracer uptake by right inguinal and by left axillary lymph nodes (empty circles; groin and axilla, respectively). Superficial: Compensatory superficial circulation (arrows). Panel C. Case 15. Deep: Reduced deep circulation bilaterally (left > right); interrupted lymphatic pathways of left limb with faint sign of compensatory superficial bilateral circulation (S: arrows); deep channel vs superficial circulation (D/S: arrows). Superficial: Faint back-flow of the superficial circulation (BF: arrow); compensatory lymphatic pathways (S: superficial circulation; D: deep circulation; arrows). Panel D. Case 16. Deep: Back-flow of the deep circulation (BF: arrows). Deep and Superficial: Compensatory anomalous superficial circulation is evident (S: superficial circulation; arrows).

Lymphoscintigraphic studies showed delay, asymmetric or absent visualization of regional lymph nodes, "dermal back-flow," asymmetric visualization of lymphatic channels, collateral lymphatic channels, interrupted lymphatic structures, and lymph nodes of the deep lymphatic system (see Figs. 1 and 2 and Table). Correlations between lymphoscintigraphic patterns and lymphatic system impairment were obtained on the basis of previously published criteria (1,9). Various patterns of TS lymphoscintigraphic studies are summarized in Figs. 1 and 2.

### DISCUSSION

Lymphoscintigraphy is a mildly invasive, easy to perform, safe, and reliable technique (9) that is very useful, especially in cases in which lymphedema or lymphatic impairment is suspected but is not yet clinically evident. Performing quantitative analysis by determination of the transport index improves the diagnostic power of lymphoscintigraphy in the very early diagnosis of lymphatic disorders.

The lymphatic system, and in particular the valves, may be underdeveloped in patients with TS and this results in childhood lymphedema. Although it has been reported that lymphedema resolves within the second year of life, there have also been reports that it can occur and reoccur at any age and it is the one clinical finding that can suggest TS on fetal ultrasonography. The combination of dysplastic or hypoplastic nails and lymphedema in infants with TS produces a characteristic sausage-like appearance to the fingers and toes (1).

Lymphoscintigraphic studies of these 15 patients reported here demonstrated various pathological features, consisting of delayed lymphatic drainage, asymmetric or absent visualization of regional lymph nodes, "dermal back-flow," asymmetric visualization of lymphatic channels, collateral lymphatic channels, interrupted lymphatic structures, and lymph nodes of the deep lymphatic system. A normal lymphoscintigraphic pattern was observed in 3 patients.

These results allowed us to identify congenital aplasia or hypoplasia of the peripheral lymphatics, and congenital lymphatic valvular incompetence, which was usually associated with megalymphatics (Table and Figs. 1,2).

We staged our TS patients according to the Consensus Document of the International Society of Lymphology (10). Only 4/18 patients presented Stage I and Stage II, pointing to clinical evidence of lymphedema. As expected, the lymphoscintigraphic study of these patients demonstrated a severe pattern characterized by interrupted lymphatic structures, severely reduced tracer uptake by inguinal lymph nodes, and back-flow mainly affecting the deep circulation with various degrees of compensatory lymphatic superficial circulation, suggesting lymphatic aplasia. The remaining 14/18 TS patients were Stage 0. The lymphoscintigraphic pattern was normal in 3 patients, as was not unexpected, while in the other 11 patients we detected various pathologic patterns suggesting different degrees of lymphatic impairment.

We may expect this latent or sub-clinical condition of lymphatic dysplasia likely to eventually turn into overt lymphedema, although the timing of lymphedema onset cannot be pre-determined.

Recently published Guidelines for TS (1-3), with reference to the National Lymphedema Network (<http://www.lymphonet.org>) and to the Consensus Document of the International Society of Lymphology (14) state that specific care is recommended only for TS patients who present clinically evident lymphedema.

In conclusion, early diagnosis of lymphedema during the neonatal age or childhood is important in order to prevent its rapid evolution and sequelae. Lymphoscintigraphy allowed us to distinguish lymphatic pathology from non-lymphatic causes of edema, and lymphoscintigraphy can detect lymphatic system impairment even in the absence of clinical evidence of lymphedema. Our results

seem to suggest that lymphoscintigraphic evaluation in the early diagnostic work-up of patients with TS should be included to obtain a pre-clinical diagnosis of lymphedema in order to establish preventive management in an attempt to delay the onset of lymphedema and to reduce the progression of lymphatic impairment.

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**Carlo Bellini, MD, PhD**  
**Dipartimento di Pediatria**  
**Università di Genova**  
**Istituto G. Gaslini,**  
**Largo G. Gaslini, 5**  
**16147 Genova, Italy**  
**Tel: +39 10 5636601**  
**Fax: +39 10 3770601**  
**Email: carlobellini@ospedale-gaslini.ge.it**