Assessment of leg oedema by dynamic lymphoscintigraphy with intradermal injection of technetium-99m human serum albumin and load produced by standing

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Abstract. This study was a preliminary evaluation of the utility of dynamic lymphoscintigraphy with technetium-99m human serum albumin (HSA) and a load produced by standing in the assessment of lymphatic dysfunction in patients with leg oedema. The 71 subjects investigated included 53 patients with lymphoedema, six with venous occlusion alone and five with lymphovenous occlusion, as well as seven normal subjects. After intradermal injection of ^{99m}Tc-HSA into an interdigital space in each foot, dynamic scintigrams were recorded with the patient supine for 15 min. The subjects then stood in place and images were recorded for an additional 15 min. Relative changes in lymphatic tracer transport before and after standing were analysed on time-activity curves (TACs). This test was compared with a conventional test in a supine position in six patients with lymphoedema, and was repeated in five other patients with lymphoedema. It was found that in the normal limbs, a standing load activated tracer transport to the draining lymphatic vessels, resulting in a rapid stepwise increase in tracer activity, large spiking waves and a decreasing phase following a peak in tracer activity on TACs. In 59 lymphoedematous limbs, including some with a mild form of oedema without morphological abnormalities on scintigrams, this load failed to induce a sufficient activation of tracer transport, and the frequencies of each of the three normally appearing changes described above significantly decreased compared with those in the 14 normal limbs ($P<0.0001$, $P<0.01$ and $P<0.0001$, respectively). In addition, there were significant reductions in the relative increases in maximum activity and clearance times after standing (both $P<0.0001$). These abnormalities significantly correlated with the grade of severity of oedema. Six limbs with lymphovenous occlusion showed significant reductions in tracer transport compared to six limbs with venous occlusion. Lymphatic dysfunction was accentuated more by this test than by the conventional test, and repeated tests showed consistent results in the same individuals. It is concluded that under a standardized load, this quick test seems of value in providing a sensitive and objective assessment of lymphatic dysfunction in the lower limbs, and is also advantageous for image interpretation since accelerated tracer transport clearly visualizes compromised lymphatics. This test may also be helpful in distinguishing purely venous oedema from mixed lymphovenous disease.

Keywords: Lymphoscintigraphy – Lymphoedema – Leg oedema – Technetium-99m human serum albumin – Lymphatic flow

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Introduction

Lymphoscintigraphy is an effective means for assessing lymphatic function based on the physiological transport of radiotracers [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13]. Lymphoscintigraphic evidence of lymphatic dysfunction in the lower limbs is usually judged from abnormal radiotracer distributions and/or delayed or absent lymphatic flow [2, 5, 8, 11, 13]. However, difficulties and uncertainties in image interpretation frequently arise because of asymmetric lymphatic tracer transport and a decrease in activity [3, 4, 7, 11]. Because of slow lymphatic flows, it takes a long time to image draining lymphatics in affected limbs [3, 4, 6]. To help overcome these problems, active or passive muscular exercises in combination with quantitative analysis of tracer activity have been applied [7, 9, 10, 11]. However, these studies have often shown significant variance and overlap in normal

and affected limbs, probably owing to poorly standardized effects of muscle pump action [3, 4, 7]. Strictly standardized exercises with walking or massage seem difficult, especially for patients with severe oedema and cutaneous thickening.

To more simply and reliably detect lymphatic dysfunction, we devised a dynamic lymphoscintigraphic test using a load involving standing from a supine position. This load is expected to activate lymphatic tracer transport by muscle pump action during the short period of standing up and by stimulation of intrinsic lymphatic contraction associated with elevated venous hydrostatic pressure [14, 15]. Comparison of lymphatic tracer transport before and after standing may accentuate lymphatic dysfunction in affected limbs. The purpose of this study was to perform a preliminary evaluation of the efficacy of this test in providing sensitive and objective assessments of lymphatic dysfunction in patients with leg oedema.

Materials and methods

Study population. The study population comprised a total of 64 patients with leg oedema [35 men and 29 women aged 19–82 years (mean±SD 63±9)] and seven normal subjects [six men and one woman aged 48–72 years (mean±SD 59±7)] who were free of leg oedema and lymphadenopathy and had no history of phlebitis or venous varices (Table 1). Of the 64 patients, 57 had unilateral leg oedema, and the remaining seven had bilateral leg oedema. The duration of the oedema varied from a few days to 13 years, with a median of 2.1 months. Oedema was classified into three grades according to clinical severity: nine limbs showed grade 1 oedema (pitting oedema without a tendency towards spreading), 42 limbs showed grade 2 oedema (swelling with a tendency towards spreading) and the remaining 20 limbs showed grade 3 oedema (non-pitting oedema with cutaneous discolouration and thickening indicative of tissue fibrosis and/or hyperkeratosis) (Table 2). Regarding the causes of leg oedema, 53 patients had primary lymphoedema (n=2), idiopathic lymphoedema (n=4) or secondary lymphoedema with causes extrinsic to the lymphatics (n=47). Six patients had venous occlusion alone, and the remaining

Table 1. Study populations

Patient group and causes of leg oedema	No. of patients	Male	Female	Age (range; mean±SD)	No. of patients who underwent contrast lymphangiography
1 Normal subjects	7	6	1	48–72; 59±7	Not performed
2 Patients with lymphoedema	53	30	23	19–82; 59±7	35
Primary lymphoedema	2	2	0	19–33; 26±9	Not performed
Idiopathic lymphoedema	4	2	2	47–61; 53±6	4
Secondary lymphoedema	47	26	21	49–82; 65±6	31
After lymphadenectomy for pelvico-inguinal neoplastic involvement	30				15
After lymphadenectomy for benign pelvico-inguinal diseases	1				1
Untreated pelvico-inguinal neoplasms and/or lymphadenopathies	6				6
After radiation therapy following resection of primary pelvic neoplasms and pelvico-inguinal lymphadenopathies	4				3
Traumatic scarring in the pelvico-inguinal region	4				4
Recurrent cellulitis in the inguinal region	2				2
3 Patients with venous occlusion alone	6	3	3	58–77; 64±6	6
Acute deep venous thrombosis	1	0	1	58	1
Chronic deep venous thrombosis with thrombophlebitis	5	3	2	58–77; 64±6	5
4 Patients with mixed occlusions of venous and lymphatic vessels	5	2	3	42–64; 57±9	4
Inguinal neoplastic involvement or simultaneous pelvico-abdominal lymphadenopathy and deep venous thrombosis	4	1	3	57–64; 57±9	4
Klippel-Trenaunay-Weber syndrome	1	1	0	42	Not performed

Table 2. Results of time-activity curve analysis in patients with leg oedema

Patient group	Stepwise increase (pos./ total limbs)	Large spiking waves (pos./ total limbs)	Decreasing phase (pos./ total limbs)	Relative increase in maximal activity (RIMA)	$T_{1/2}$ (min)
1 Patients with lymphoedema					
Primary lymphoedema					
Affected limbs (*n*=3)					
Grade 3 (*n*=3)	0/3	1/3	0/3	1.8±0.2	–
Non-affected limbs (*n*=1)	1/1	1/1	1/1	13.9	4.0 (*n*=1)
Idiopathic lymphoedema					
Affected limbs (*n*=5)					
Grade 2 (*n*=2)	1/2	0/2	0/2	2.9±0.2**	–
Grade 3 (*n*=3)	0/3	0/3	0/3	1.8±0.5****	–
Non-affected limbs (*n*=3)	3/3	2/3	3/3	9.8±0.4	4.1±1.2 (*n*=3)
Secondary lymphoedema					
Affected limbs (*n*=51)					
Grade 1 (*n*=7)	4/7*	4/7**	3/7**	3.6±0.4**	40.8±3.3**** (*n*=3)
Grade 2 (*n*=33)	5/33****	20/33****	5/33****	2.9±0.5****	102.8±30.6**** (*n*=5)
Grade 3 (*n*=11)	1/11****	3/11****	1/11****	1.9±0.2****	196 (*n*=1)
Non-affected limbs (*n*=43)	40/43	42/43	39/43	5.4±1.5	7.1±3.0 (*n*=39)
2 Patients with venous occlusion alone					
Acute deep venous thrombosis					
Affected limbs (*n*=1)					
Grade 2 (*n*=1)	1/1	1/1	1/1	5.4	3.3 (*n*=1)
Non-affected limbs (*n*=1)	1/1	1/1	1/1	5.1	6.4 (*n*=1)
Chronic deep venous thrombosis with thrombophlebitis					
Affected limbs (*n*=5)					
Grade 1 (*n*=2)	2/2	2/2	2/2	6.5±4.6	6.8±4.3 (*n*=2)
Grade 2 (*n*=2)	2/2	2/2	2/2	6.2±0.1	7.5±5.8 (*n*=2)
Grade 3 (*n*=1)	1/1	1/1	0/1	7.0	–
Non-affected limbs (*n*=5)	5/5	4/5	5/5	7.0±1.3	5.2±1.4 (*n*=5)
3 Patients with mixed occlusions of venous and lymphatic vessels					
Inguinal neoplastic involvement or simultaneous pelvico-abdominal lymphadenopathy and deep venous thrombosis					
Affected limbs (*n*=4)					
Grade 2 (*n*=4)	1/4	2/4	1/4	2.3±0.7	167 (*n*=1)
Non-affected limbs (*n*=4)	4/4	3/4	4/4	5.4±2.4	5.2±2.1 (*n*=4)
Klippel-Trenaunay-Weber syndrome					
Affected limbs (*n*=2)					
Grade 3 (*n*=2)	0/2	0/2	0/2	1.9±0.2	–
Non-affected limbs (*n*=0)	–	–	–	–	–

Grade 1, pitting oedema without a tendency towards spreading; grade 2, swelling with a tendency towards spreading; grade 3, non-pitting oedema with cutaneous discolouration and thickening indicative of tissue fibrosis and/or hyperkeratosis

*P<0.05, **P<0.01, ****P<0.0001, compared with the non-affected limbs in each group

five patients had mixed occlusions of venous and lymphatic vessels (Table 1). Contrast lymphangiography confirmed lymphatic disruption and/or the presence of inguinal and/or pelvic lymphadenopathies in a total of 45 patients. This procedure was not performed in two patients with primary lymphoedema since it is considered to be contraindicated in this disorder. In all 53 patients with lymphoedema, the absence of venous occlusion was established by radionuclide venography with technetium-99m macroaggregated albumin (^{99m}Tc-MAA) or ^{99m}Tc-human serum albumin (HSA) (*n*=31) and/or vascular Doppler ultrasonography (*n*=28), and by contrast venography (*n*=11). In six patients with venous occlusion alone, venous occlusion was confirmed by contrast venography. In four of

the five patients with mixed occlusions of venous and lymphatic vessels, the mixed occlusions were established by contrast lymphangiography and venography; the exception was a patient with Klippel-Trenaunay-Weber (KTW) syndrome in whom venous and lymphatic angiopathies were confirmed histologically. None of the 64 patients had clinical evidence of cardiac or renal dysfunctions that would cause leg oedema. Thus, 14 control normal limbs, 59 limbs with lymphoedema (7 with grade 1 oedema, 35 with grade 2 and 17 with grade 3), 6 limbs with venous occlusion (2 with grade 1 oedema, 3 with grade 2 and 1 with grade 3), 6 limbs with lymphovenous occlusion (4 with grade 2 oedema and 2 with grade 3), and 57 non-affected limbs were studied.

To compare the findings of the present tests with those of conventional lymphoscintigraphy in a supine position, five of the 53 patients with lymphoedema (two with idiopathic grade 2 oedema, and three with grade 3 oedema after lymphadenectomy for inguinal neoplastic involvement) also underwent the conventional test at an interval of 7–10 days. The conventional procedure was performed using the same protocol as for our proposed test, but with a longer total acquisition time of 60 min. Two days later, the peripheral venous pressure was measured in the dorsal veins of both feet before and after a standing load in these patients.

Furthermore, to evaluate the consistency of the findings of this test in the same individuals, another five patients with grade 2 lymphoedema after lymphadenectomy for inguinal neoplastic involvement underwent this test twice, at an interval of 6–8 days. Informed consent for all examinations was obtained from all subjects.

Lymphoscintigraphic technique. Human serum albumin (HSA) from a commercially available kit (Daiichi Radioisotope Laboratories, Ltd, Chiba, Japan) was labelled with high specific activity ^{99m}Tc-pertechnetate. Each subject was initially placed for at least 10 min in a supine position under a large field of view Anger camera with a low-energy, all-purpose collimator (Toshiba GCA-901 A, Tokyo Shibaura Electric Co. Ltd., Japan) attached to a nuclear medicine computer (Fig. 1). During this period, the patients were instructed to refrain from moving their legs so as to obtain complete relaxation of the leg muscles, and in order to prevent the effect of muscular pump action on lymphatic flow. ^{99m}Tc-HSA [dose: 37 MBq (1 mCi); volume: 0.05–0.08 ml] was then intradermally injected into an interdigital space in each foot, whether involved or uninvolved, using a tuberculin syringe and a 27 gauge needle. Within 20 min of preparation, this tracer was injected slowly and carefully to produce an intradermal injection, without subcutaneous infiltration, at high pressure, forming a superficial weal similar to that in the intradermal Mantoux tuberculin test. The use of ^{99m}Tc-HSA and intradermal injection was based on the excellent results of previous studies that had shown faster delineation of lymphatic vessels compared with that achieved using subcutaneous injection of this tracer or ^{99m}Tc-sulphur colloids [2, 5, 10]. The subjects, who remained supine, were again instructed to refrain from moving their legs to avoid irregular muscle pump action. Immediately after injection, dynamic anterior images covering both upper thighs and pelvis were recorded with an acquisition time of 10 s for 15 min, with the energy window set at 20% centred on 140 keV. After this, the subject stood up slowly within a 15-s period. No data were recorded during this period, in which the gamma camera, using external markers, was reset to replicate its position during data recording in the supine position. The same distance between collimator surface and skin was maintained. As the subjects stood up, they were advised not to move their legs so as to avoid irregular muscle pump function. Images were again recorded for 15 min in a similar fashion as in the supine position. The patients stood still for the acquisition time. Thereafter, delayed static images of distal and proximal extremities and the pelvis were taken with the patients supine between 60 and 90 min after injection, with an acquisition time of 10 min. When draining lymphatics were not well visualized, additional delayed images were obtained 2 or 3 h later.

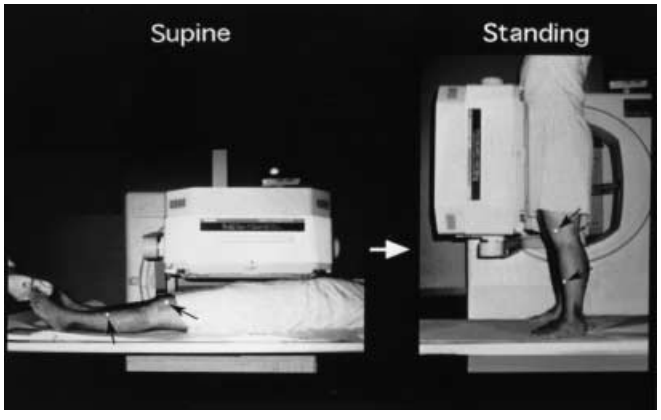

Fig. 1. Dynamic lymphoscintigraphic test using a standing load. After intradermal injection of tracer into an interdigital space in each foot in the supine position, dynamic images covering both upper thighs and pelvis are recorded for 15 min. Then, the subject stands up slowly, and the gamma camera is reset using external markers (*arrows*) to replicate its position during data recording in the supine position. Dynamic images are again recorded for 15 min. During image acquisition, the subjects are instructed not to move their legs

To assess lymphatic tracer transport, the time-activity curves (TACs), obtained from regions of interests (ROIs) manually placed over the symmetric portions of the lymphatic vessels at the level of the upper thighs were analysed [9, 10]. For the ROI setting, a summation image formed from two to five sequential frames after standing was used to depict clearly the lymphatic vessels. In cases where lymphatic vessels were not clearly visible, ROIs were placed in the medial portion of the thigh, where the draining lymphatics normally appear. TACs were produced using supplemental software in the data processor (Toshiba GMS-550U; Tokyo Shibaura Electric Co. Ltd., Japan). The final data point in the supine position and the first data point soon after standing up were connected with a line although there was actually a gap between the two points.

Evaluation of lymphoscintigraphy. Visual interpretation of the scintigrams was independently performed by two experienced nuclear medicine physicians (K.S. and N.K.), neither of whom had prior details of patient status. The presence or absence of activated lymphatic tracer transport after standing, morphological abnormalities such as obstruction of lymphatic vessels, collateral pathways and backflow into the soft tissue (e.g. tracer extravasation or diffuse activity of soft tissue), and the number of and distribution patterns in inguinal lymph nodes were assessed on the serial images by consensus. The interpretation of TACs focussed on the presence or absence of three findings indicative of activated tracer transport after standing, which are accepted as normal patterns: (a) a sharp stepwise increase in tracer activity soon after standing, (b) a large spiking wave and (c) an exponential decrease in tracer activity over time following a peak in tracer activity (Fig. 1). Of

these findings, a sharp stepwise increase in tracer activity was defined as one in which the tracer activity was more than threefold the maximal activity of the tracer observed before standing, and a large spiking wave as one in which the amplitude was more than threefold that of waves before standing. As quantitative parameters on TACs, the relative increase in maximal activity (RIMA), as expressed by the ratio of maximal activity after standing to that before standing, and the real half clearance time ($T_{1/2}$) of a peak in activity in a decreasing phase after standing, were estimated. To calculate $T_{1/2}$, a decreasing phase was fitted to a mono-exponential curve by a method of least squares. The correlation coefficient for the best fit in the logarithmic regression line of all clearance curves exceeded 0.902 (average 0.931±0.027).

Statistical analysis. Data in respect of RIMA, $T_{1/2}$ and peripheral venous pressure were expressed as means ± SD, and differences in the means between the groups were assessed using unpaired or paired Student's *t* tests. Fisher's exact or chi-square tests on a contingency table were used for comparison between the groups with regard to the difference in frequency of each of the above three changes on TACs that were considered indicative of activated tracer transport after standing. In these analyses, a *P* value less than 0.05 was regarded as significant. The linear correlation between RIMA and $T_{1/2}$ values of normal and non-affected limbs and patient age was assessed by a linear regression analysis using commercially available software (StatView 4.02 SE + Graphics; Abacus Concepts, Berkeley, Calif.). A *P* value less than 0.05 was considered significant for each correlation coefficient (*r*).

Results

In normal control limbs, the standing load accelerated the tracer transport upward to a single or two bands of the draining lymphatic vessels along the medial aspect of the bilateral thighs (Fig. 2). The inguinal and pelvic lymph nodes symmetrically appeared on both sides on the delayed scintigrams. TACs appeared nearly identical on both sides, with sharp stepwise increases in tracer activity, large spiking waves and a decreasing phase following a peak in activity appearing in all limbs after standing, with the exception of one limb that lacked large spiking waves because of the presence of several high-amplitude waves before standing (Fig. 2, Table 3).

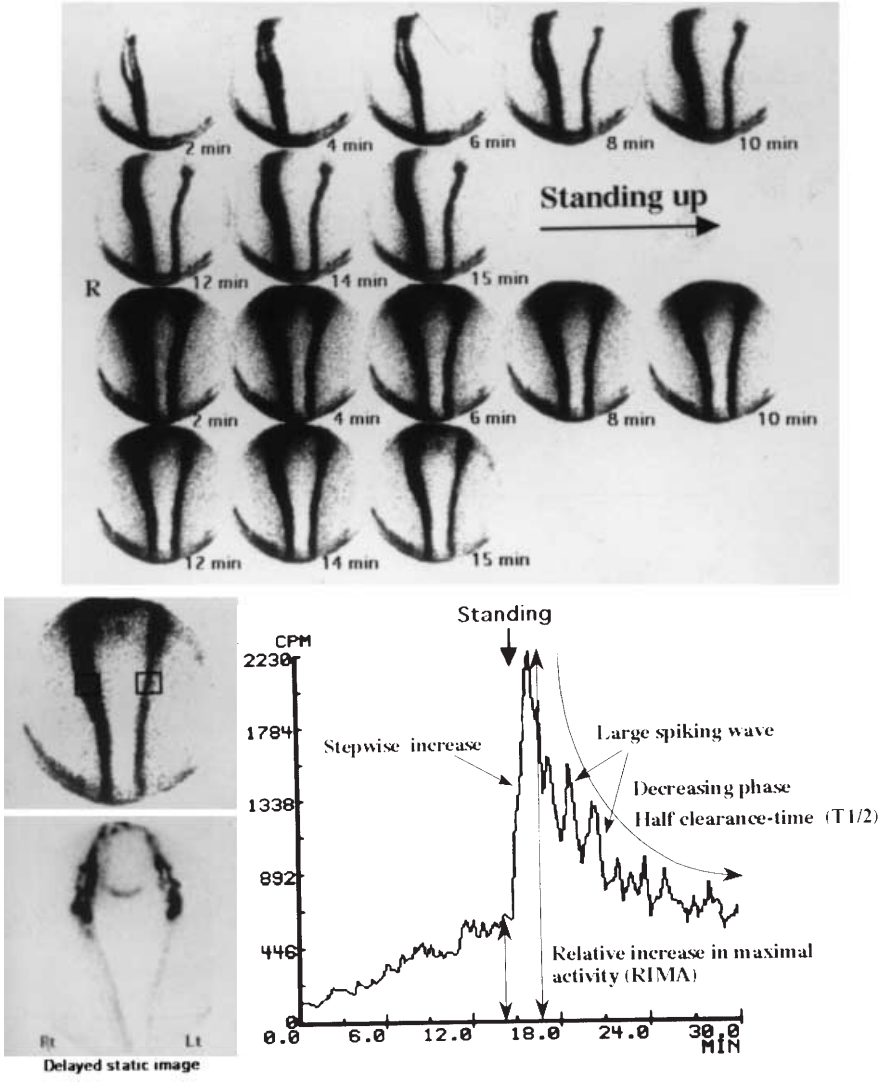

Fig. 2. A normal subject (48-year-old male). *Top:* Dynamic ^{99m}Tc-HSA lymphoscintigraphy using a standing load, showing prominent acceleration of tracer transport upward to the draining lymphatic vessels in the bilateral thighs after standing. *Bottom:* The setting of ROIs of symmetric portions of the lymphatic vessels at the upper level of the thighs (*left*) and the TAC obtained from the right ROI (*right*). Note three prominent changes after standing that are indicative of activated lymph flow, i.e. a sharp stepwise increase in tracer activity, a large spiking wave, and a decreasing phase following a peak in activity. The relative increase in maximal activity (RIMA), expressed as the ratio of maximal activity after standing to that before standing (↑), and the half clearance time ($T_{1/2}$) of a peak in activity at the decreasing phase in this normal limb were 3.4 and 3.2 min, respectively

Table 3. Comparison of the results of TAC analysis between control normal limbs and affected and non-affected limbs in patients with leg oedema

Group	Stepwise increase (pos./ total limbs)	Large spiking waves (pos./ total limbs)	Decreasing phase (pos./ total limbs)	Relative increase in maximal activity (RIMA)	$T_{1/2}$ (min)
Control (*n*=14)	14/14	13/14	14/14	6.2±1.7	6.6±3.1 (*n*=14)
Non-affected limbs (*n*=57)	54/57	53/57	53/57	6.1±2.1	6.5±2.9 (*n*=53)
Affected limbs (*n*=71)					
Lymphoedema (*n*=59)	11/59****	28/59**	9/59****	2.7±0.7****	92.5±53.6 **** (*n*=9)
Grade 1 (*n*=7)	4/7*	4/7	3/7**	3.6±0.4**	40.8±3.3**** (*n*=3)
Grade2 (*n*=35)	6/35****	20/35*	5/35****	2.9+0.5$^{****, a}$	102.8±30.6$^{***, b}$ (*n*=5)
Grade 3 (*n*=17)	1/17****	4/17***	1/17****	1.9±0.2$^{****, c}$	196 (*n*=1)
Venous disease (*n*=6)	6/6	6/6	5/6	6.3±2.1	6.4±4.0 (*n*=5)
Lymphovenous disease (*n*=6)	1/6****	2/6*	1/6****	2.2±0.6****	167 (*n*=1)

Grade 1, pitting oedema without a tendency towards spreading; grade 2, swelling with a tendency towards spreading; grade 3, non-pitting oedema with cutaneous discolouration and thickening indicative of tissue fibrosis and/or hyperkeratosis

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ compared with control normal limbs

$^{a}P<0.01$ comparing grade 1; $^{b}P<0.05$ comparing grade 1; $^{c}P<0.0001$ comparing grade 2

RIMA ranged from 3.6 to 10.1, and $T_{1/2}$ from 2.8 to 11.2 min, with no consistent right to left differences in these values. All the 57 non-affected limbs of the 64 patients showed similar findings to those in the control limbs; overall there were no significant differences in the frequencies of each of the three normally appearing changes on TACs after standing, or in RIMA and $T_{1/2}$ values (Table 3). There was a significant linear dependency between $T_{1/2}$ in control and non-affected limbs and the ages of the subjects, as y=44.149+2.541x (n=67, r=0.706, P<0.0001), although no significant correlation was noted with respect to RIMA values.

In the majority of the lymphoedematous limbs, tracer transport on scintigrams accelerated after standing, but to lesser degrees than the acceleration in control and non-affected limbs. In seven limbs with grade 2 or 3 oedema, the draining lymphatic vessels, which had not been visualized before standing, became fairly visible after this load. However, no migration of tracer from the injected area was seen in another two limbs with grade 3 oedema. Including these two limbs, morphological abnormalities appeared in 47 (79.6%) of the 59 affected limbs on scintigrams. The number of inguinal lymph nodes visualized was decreased in those patients who had undergone lymphadenectomy or had inguinal neoplasms/lymphadenopathies. No morphological abnormalities, however, were seen in the remaining 12 (20.3%) affected limbs with grade 1 or 2 oedema. The most delayed scintigrams did not yield additional information, except for further accumulations of tracer in the soft tissues. On TACs, the differences in tracer transport between the affected and the non-affected limbs became much clearer after standing, and the three normally appearing changes were often absent in the affected limbs (Figs. 3, 4). Of the 12 affected limbs without morphological abnormalities, 11 (91.6%) lacked at least one of the three normal changes (Fig. 4). The frequencies of the three changes, and RIMA and $T_{1/2}$ values, were lower in the affected limbs than in the non-affected limbs and control limbs, and varied according to the grade of oedema (Tables 2, 3). Overall, there were significant differences in the frequencies of each of the three changes, as well as RIMA and $T_{1/2}$, between the 59 affected limbs and control normal limbs (P<0.01 to P<0.0001) (Table 3). The frequency of a stepwise increase was significantly lower in grade 2 and 3 oedema than in grade 1 oedema (both P<0.05), and the frequency of large spiking waves was significantly lower in grade 3 oedema than in grade 2 oedema (P<0.05). RIMA and $T_{1/2}$ also decreased significantly in accordance with the severity of the oedema. Even in a limb with grade 1 oedema that did not show morphological and TAC abnormalities, $T_{1/2}$ was prolonged beyond 12.8 min (the mean value + 2SD in normal control limbs).

The six limbs with mixed lymphovenous occlusions showed acceleration of tracer transport after standing, but to lesser degrees compared with control and non-affected limbs (Fig. 5). Two limbs with grade 3 oedema associated with KTW syndrome, however, showed persistent stasis of the tracer at the injected site (Tables 2, 3). Although no morphological abnormalities were seen in the two affected limbs, the overall frequencies of each of the three normally appearing changes and RIMA in all six limbs were significantly reduced compared with those in control and non-affected limbs (P<0.0001 in each case) (Table 3).

All six limbs with venous occlusion alone showed image findings similar to those in control limbs (Fig. 6). TAC patterns were also similar, except that a decreasing phase was absent in one limb with grade 3 oedema and

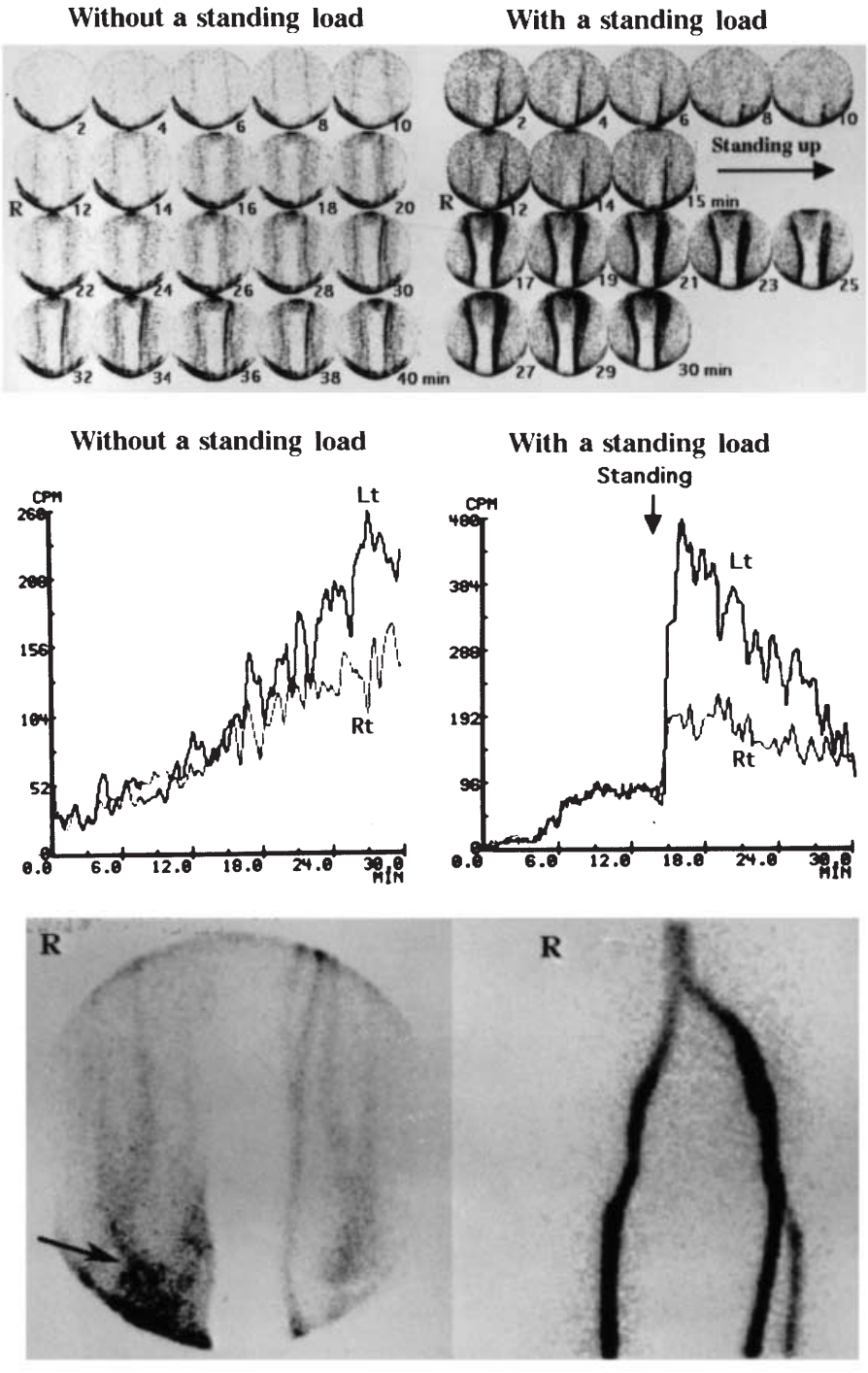

Fig. 3. Comparison of a conventional lymphoscintigraphy in a supine position and a test using a standing load in a 63-year-old male with grade 2 lymphoedema in the right leg. *Top:* Lymphatic vessels are faintly seen in the affected right limb on dynamic images in a conventional test (*left*). However, in a test using a standing load (*right*), the tracer transport is markedly accelerated after standing and the draining lymphatic vessels are more clearly visualized in both limbs. *Middle:* TACs in a conventional test showing only slight differences in tracer transport between the affected right and non-affected left limbs. In contrast, on TACs in a test using a standing load, the difference is more accentuated. The three changes after standing that are indicative of normally activated lymph flow (Fig. 1), are less evident in the right limb than in the left limb. RIMA and $T_{1/2}$ values were 2.1 and 21.2 min in the affected right limb and 4.1 and 6.2 min in the non-affected left limb, respectively. *Bottom:* Delayed scintigram (*left*) shows slight dermal backflow. ^{99m}Tc-MAA venography (*right*) shows no venous obstruction

chronic thrombophlebitis. A $T_{1/2}$ of 3.3 min in one limb with acute venous occlusion due to thrombosis was the fastest among all limbs studied (Fig. 6). Overall, there were no significant differences in the frequencies of each of the three normally appearing changes, or in RIMA and $T_{1/2}$ (n=5), between these six affected limbs and control limbs (Tables 2, 3). However, compared to the six limbs with lymphovenous occlusions, the frequencies of each of the three changes were significantly greater (P<0.01, P<0.05 and P<0.01, respectively), and the RIMA value significantly higher (P<0.01).

In a comparative study with the conventional test in five patients with lymphoedema, the draining vessels were better visualized after standing in all the affected limbs, and abnormal lymphatic tracer transport in these limbs was more accentuated on TACs (Fig. 3). In these patients, peripheral venous pressure increased significantly from 21.7±4.3 to 32.9±4.1 cmH_2O after standing

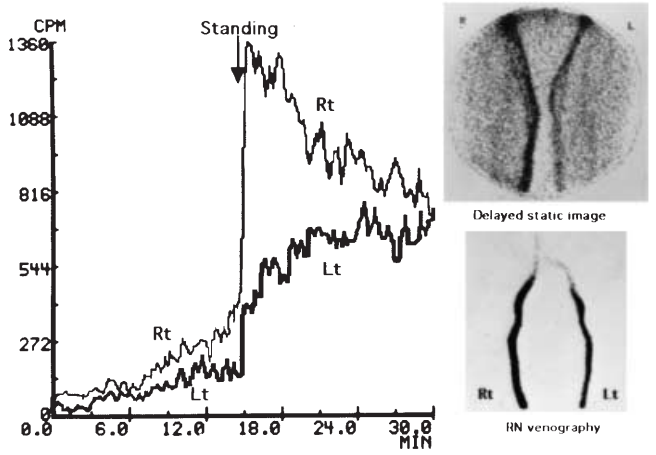

Fig. 4. A 65-year-old female who developed mild lymphoedema of grade 1 in the left leg after inguinal lymphadenectomy. TACs (*left*) do not show a decreasing phase of tracer activity after a standing load in the affected left limb. RIMA values were 3.2 in the affected left limb and 3.5 in the non-affected right limb. No obstructive findings in the vessels are seen either on the delayed lymphoscintigram (*right, top*) or on ^{99m}Tc-99m MAA venography (*right, bottom*)

in the five non-affected limbs, and from 22.4±5.2 to 32.0±4.5 cmH_2O in the five affected limbs (both P=0.0001).

The repeated tests in another five patients with lymphoedema showed consistent findings for both images and TAC patterns. There were no significant differences between the two measurements of RIMA and $T_{1/2}$ in the five affected limbs (3.1±1.3 vs 3.2±1.6, n=5, and 91.7±33.2 min vs 90.4±38.5 min, n=4, respectively), or in the five non-affected limbs (6.1±2.2 vs 6.2±1.8, n=5, and 6.6±4.3 min vs 6.5±4.6 min, n=5, respectively).

Discussion

The proposed test clearly demonstrated activated lymphatic transport in normal limbs after standing, and the failure of sufficient activation in lymphoedematous limbs. The TAC analysis enabled us to detect retarded tracer transport even in a mild form of oedema without morphological abnormalities on scintigrams. The good correlations between the results of TAC analysis and the grade of severity of lymphoedema, and the consistent results in the repeated tests in the same individuals indicate that this test using a standardized load may be acceptable for the objective assessment of lymphatic dysfunction. Our test seems superior to the conventional test in a supine position for the detection of lymphatic dysfunction, because the standing load more effectively and quickly accentuates reduced tracer transport and any anatomical compromise of the lymphatics.

The three normally appearing changes after standing on TACs may reflect not only the effect of muscular

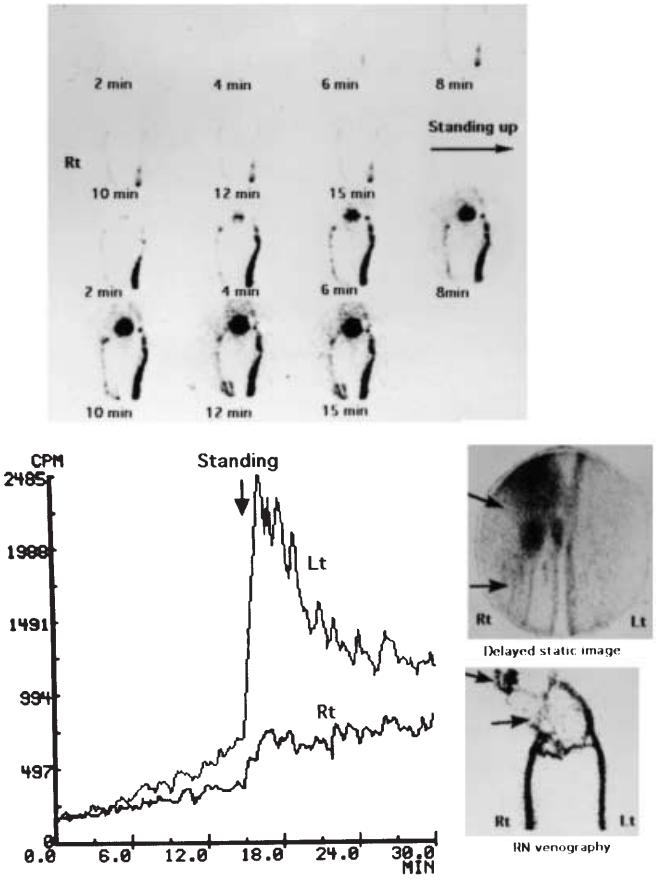

Fig. 5. A 72-year-old male with mixed occlusions of venous and lymphatic vessels and with grade 3 oedema in the left leg associated with inguinal metastatic lymph nodes. *Top:* On dynamic images, the tracer transport is accelerated after a standing load and the draining lymphatic vessels are better visualized in the bilateral limbs. *Bottom:* TACs (*left*) showing the absence of large spiking waves and a decreasing phase of tracer activity after standing in the affected right limb. RIMA values were 1.2 in the affected right limb and 3.2 in the non-affected left limb. Delayed scintigram (*right, top*) showing collateral lymphatic vessels and dermal backflow (*arrows*). ^{99m}Tc-MAA venography (*right, bottom*) showing venous obstruction in the right iliac vein with collateral vessels (*arrows*)

pump action during adoption of a standing position but also that of the elevated hydrostatic pressure in this position. Lymphatic vessels dilate owing to elevated hydrostatic pressure, resulting in an increase in tracer activity. Isolated lymphatic vessels have an intrinsic contraction capacity such that they are likened to lymphatic pacemaker cells. An increased lymphatic outflow pressure normally activates this capacity to transport the lymph upward, even in the absence of muscular contractions or arterial pulsations [12, 14, 15, 16]. Activated lymphatic contraction in a standing position effectively drains the increased interstitial lymph fluid transferred from the peripheral venous capillaries owing to the increased hydrostatic pressure, and contributes to prevention of leg oedema [12, 14, 15, 16, 17]. The large spiking waves ob-

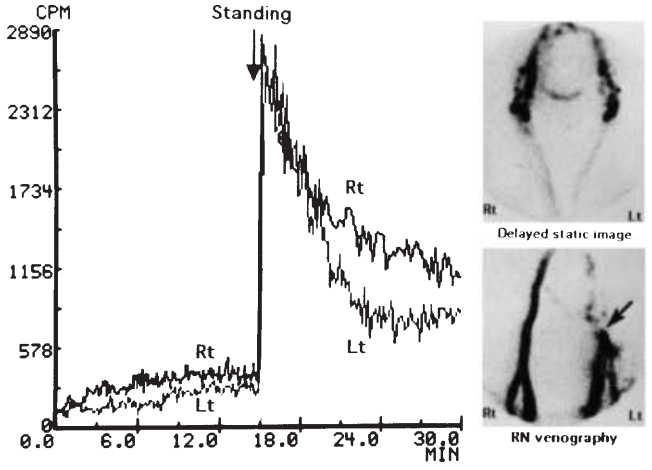

Fig. 6. A 57-year-old female with acute venous thrombosis and grade 2 oedema in the left leg. TACs (*left*) showing the three changes after standing that are indicative of normally activated lymph flow (Fig. 1) in the bilateral limbs. The clearance time ($T_{1/2}$) after standing is rather faster in the affected right limb (3.3 min) than in the non-affected left limb (6.4 min). Delayed scintigram (*right, top*) shows no abnormality. ^{99m}Tc-MAA venography (*right, bottom*) shows venous occlusion in the left femoral vein (*arrow*)

served after standing seem to reflect this activated, intrinsic lymphatic pump activity [9, 10, 13]. A decreasing phase after standing also seems to reflect this activation, and may result from the rapid clearance of the tracer from the ROIs toward upper regions. A sharp stepwise increase soon after standing may reflect prompt dilatation of the lymphatics and a prompt increase in lymphatic inflow into the ROIs. By contrast, the limbs with lymphatic interruptions may fail to show sufficient dilatation of lymphatic vessels owing to compression by increased interstitial lymph fluid and/or fibrosis. The intrinsic lymphatic pump activity also may deteriorate because of lymphatic fibrosis, stenosis or valvular incompetence [6, 11, 16, 18]. These lymphatic dysfunctions seem to result in absence of some of the above three changes and a decline in RIMA and $T_{1/2}$ values on TACs after standing. Further, the difference in tracer transport between affected and non-affected limbs is increased after standing, as this study shows.

To detect lymphatic dysfunction, this study focussed on the relative changes in tracer transport before and after standing as registered on TACs. This assessment seems indispensable for the sensitive detection of mild lymphatic dysfunction, as retarded tracer transport could be identified in some affected limbs without signs of morphological abnormalities. Lymphatic function is often reported to be impaired before morphological changes appear on scintigrams [3, 9]. The proposed test using a standardized load appears to permit the objective assessment of lymphatic tracer transport. The correlation between $T_{1/2}$ values and age in the normal and non-affected limbs seems to reflect clearly the deterioration in intrinsic lymphatic pump function associated with aging [19]. The results of TAC analysis correlated well with the severity of lymphoedema. Lymphatic tracer transport has been quantified using absolute values of the clearance time of tracer activity from the injected sites or the arrival times at major lymph nodes [3, 4, 6, 7, 11]. However, these studies showed some difficulties in discriminating abnormal from normal lymph flows because of the wide variance in the results [3, 4, 7, 11]. Asymmetric injection of tracer and dilution of tracer activity in oedematous legs would greatly influence the absolute values. The proposed test, assessing relative changes before and after standing, does not necessarily require strictly symmetric injection of the tracer in both feet. The fact that it allows reliable assessment is demonstrated by the consistent results in repeated tests in the same individuals.

This test distinguished purely venous oedema from lymphoedema and mixed lymphovenous diseases, except in one case of long-standing thrombophlebitis [1, 6, 19, 20]. In oedematous limbs with venous occlusion only, lymph flow is reported to be normally preserved or rather activated to transport the increased interstitial fluid associated with venous occlusion [1, 6, 19, 20, 21]. The fastest $T_{1/2}$ value, in the patient with acute venous thrombosis, may reflect this activated lymphatic function. However, as seen in one exceptional case of long-standing venous insufficiency, lymph flow may be simultaneously disturbed by damage or fragmentation of dermal lymphatic networks associated with recurrent phlebitis, and by compression of lymphatics by increased interstitial fluid and cutaneous thickening [7, 11, 19, 22, 23]. In such cases, the condition might not be easily distinguished from pure lymphoedema. Scintigraphic assessment of lymphatic function in limbs with venous pathology, however, is important, since the cycle of oedema, infection and fibrosis will cause further deterioration in lymph drainage if lymphatics are additionally affected [19].

It may be possible to shorten the examination time of the proposed test, since the most delayed scintigrams did not provide useful additional information except regarding further extravasation of tracer into soft tissues. This is advantageous in practice as the enhanced tracer transport may be caused not only by the effect of a standing load, but also by the rapid migration of intradermally injected ^{99m}Tc-HSA to drainage vessels. The intradermally injected HSA with a small particle size of approximately 4 nm is rapidly taken up by the abundant intradermal, initial lymphatic vessels, and by high pressure in the dense connective tissue [3, 9, 10]. This tracer is commercially available for easy labelling and routine use [9, 10], and radiation doses are minimal compared with other radiocolloids [3, 24]. This test is also beneficial for image interpretation, as anatomical integrity or compromise of the lymphatics in the affected limbs could be more clearly and quickly evidenced. It may prevent overestimation of lymphatic interruptions in severely lymphoedematous limbs, since in sev-

eral of our patients the loading manoeuvre succeeded in showing the lymphatic vessels, which had not been visible during supine position imaging.

In conclusion, by assessing relative changes on TACs before and after a standing load, abnormal lymphatic tracer transport was perceptible even in a mild form of lymphoedema without morphological abnormalities. Consistent results on TACs were noted in the repeated tests in the same patients, and the quantification of the TACs revealed that lymphatic function was variably affected in individual patients even when they had the same grade of leg oedema, although the results overall correlated with the clinical severity of the oedema. It also showed deterioration of lymphatic function with aging in the non-affected limbs. The suggested test is useful for sensitive and objective assessment of lymphatic dysfunction. It also seems beneficial for image interpretation, since accelerated tracer transport clearly and quickly showed compromise of the lymphatics, and may be helpful for distinguishing purely venous oedema from mixed lymphovenous disease.

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