

EFFECT OF LATERALIZATION AND HANDEDNESS ON THE FUNCTION OF THE LYMPHATIC SYSTEM OF THE UPPER LIMBS

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ABSTRACT

In the upper limbs (UL), lateralization or handedness coincides with functional (and/or anatomical) asymmetries. Scintigraphic techniques were used to investigate the function of the UL lymphatic system and to determine whether significant differences exist between right and left limbs. With limbs at rest, ^{99m}Tc-labeled HSA nanosized colloids were injected intradermally into the forearms in 19 volunteers. Activities in the axillary nodes were recorded 1 minute later, then every 20 minutes up to 100 minutes later and reported as per ten thousands of injected activity. When comparing right injections to left injections, no significant difference was found using an unpaired statistical test. However, with paired t-tests, axillary node activity (mean ± SD) was significantly higher when the right limb was injected than when the left limb was injected, both overall (n=19) (at 100 minutes: 454 ± 88 versus 299 ± 45; p=0.014) and when the 13 right-handed cases were analyzed separately (501 ± 116 versus 285 ± 65; p=0.004). No difference was found for the six left-handed volunteers. Our results demonstrate, at least in the right handed subjects, the functional asymmetry of the superficial lymphatic system of the right and left upper limbs.

Keywords: handedness, lymphatic function, lymphoscintigraphy, intradermal, axillary nodes

Normal asymmetries between the right and left parts of the human body (and/or between males and females) are well documented for some human systems, including blood pressure (1,2), transepidermal water loss (3,4), muscle structure and weight (5-8), bones (9,10), and cutaneous microvascular flow (11).

Lymphoscintigraphy using ^{99m}Tc-labeled colloids is a simple and relatively nontraumatic way to investigate the lymphatic system. The question of lateralization and its possible influence on the results of lymphoscintigraphic investigations has already been raised in one study following subcutaneous injections of ^{99m}Tc-labeled colloids (12). An effect of aging on the results after subcutaneous injection of labeled colloids in the fifth or sixth intercostal space of the anterior thoracic wall was found significant for the injections in the right side but not for the injections in the left side.

Using scintigraphic techniques after injection of ^{99m}Tc-labeled colloids, we assessed whether functional lymphatic asymmetry could be observed between right and left upper limbs.

MATERIALS AND METHODS

Nineteen young healthy male volunteers (mean age=23 years, range: 18-28) were examined. The participants had no history of

upper limb osteoarticular or lymphatic lesions. Candidates with any disease such as psoriasis or diabetes were also excluded from the study, as were those who played sports such as basketball because of their possibly having undiagnosed lesions of the lymphatic system. The investigational protocol was approved by the Institutional Ethical Committee, and all volunteers gave their written informed consent.

Thirteen participants reported being “right-handed,” and six said that they were “left-handed.” Handedness was more precisely established using the Edinburgh Inventory (13). Three of the six “left-handed” subjects were subsequently classified as “pure” left-handed, and three showed one ambidextrous-ambilateral component. Twelve of the thirteen “right-handed” volunteers were also classified as “pure” right-handed and one showed one ambidextrous-ambilateral component.

Injections were standardized. Pure intradermal injections of the same volume (0.2 ml), quantity (one-tenth of one vial) and activity (0.5 mCi or 17 MBq as a mean activity per injection) of ^{99m}Tc -labeled human serum albumin (HSA) nanosized colloids (Nanocoll R) were performed in the anterior (ventral) middle part of each forearm by a well-trained physician (PB) using a 29-gauge micro-fine syringe. The quality of the intradermal injection was visually assessed by the appearance of an orange skin aspect.

Activities were recorded using a single-head, single-photon gamma camera (SophyCam R, Sopha, Belgium or MaxiCam, GE, Belgium) equipped with a parallel-hole, all-purpose low-energy collimator at the primary site of administration just after injection (dynamic acquisition: 10 frames matrix 64x64x16 or word mode, 2 seconds each) and at the level of the axillary regions shortly afterwards at 1 (T+1) and 20 (T+20), 40 (T+40), 60 (T+60), 80 (T+80) and 100 (T+100) minutes later (static acquisition, frame matrix 128x128x8 or byte mode, 60 seconds each). Acquisition readings were

performed with the participant seated facing the gamma camera field with the limbs at rest, both before the injection was performed and during the reading period.

Activity readings (AT+x) were corrected for physical decay and background, normalized for the acquisition parameters, and expressed as per ten thousands of the activity injected at T=0 (AT0) in the corresponding forearm using the formula: (AT+x) multiplied by 10,000 and divided by AT0.

Numeric values at the given timepoints after injection were compared using a paired T-test (two-tailed Wilcoxon signed-rank test) for the comparisons of the right to left forearm in the same persons and a unpaired chi-square test for the unpaired comparisons of the right values to the left ones.

RESULTS

Mean activities and the results of statistical analysis are presented in *Table 1*.

When we considered the right and the left upper limbs as two different unpaired series, mean right axillary node (AxN) activity was 1.5 times greater than mean left AxN activity, although this difference was not statistically significant. However, when we analyzed the series as a whole and used a paired T-test to compare the right upper limb to the left one, the difference between the 19 paired right and left AxN activities was statistically significant ($p < 0.02$ for any time from 20 to 100 minutes after injection).

We then considered the influence of handedness on AxN following right forearm versus left forearm injections. In the group of the 13 right-handed men, the mean right AxN activity was again significantly higher (two times greater) than left AxN activity ($p < 0.006$ for any time from 20 to 100 minutes after injection). In contrast and although the mean activity for the right AxNs was higher than that of the left, in the group of the six left-handed participants, right and left AxN activities did not differ significantly as determined by the paired T-test.

TABLE 1
Axillary Node Activities* as a Function of Time after Injection and Handedness

Time after injection in minutes	Total cases		"Right-handed" cases		"Left-handed" cases	
	Right	Left	Right	Left	Right	Left
1	19±8 (1-107)	15±5 (0-57)	23±12 (0-107)	17±6 (0-53)	10±10 (0-59)	11±9 (0-57)
	[NS]		[NS]		[NS]	
20	235±55 (1-765)	142±29 (10-441)	277±76 (30-765)	142±40 (10-441)	159±71 (1-482)	141±44 (22-276)
	[0.011]		[0.005]		[NS]	
40	339±68 (16-945)	209±33 (26-530)	373±88 (50-945)	202±47 (26-530)	278±109 (16-633)	221±42 (81-342)
	[0.008]		[0.004]		[NS]	
60	409±78 (25-1007)	247±45 (36-687)	428±101 (69-1002)	241±65 (36-687)	370±136 (25-766)	259±53 (109-392)
	[0.002]		[0.003]		[NS]	
80	463±88 (32-1210)	272±46 (48-680)	494±115 (87-1210)	263±65 (48-680)	401±145 (32-829)	289±56 (137-439)
	[0.002]		[0.003]		[NS]	
100	454±88 (43-1370)	299±45 (50-772)	501±116 (101-1370)	285±65 (64-772)	367±136 (43-874)	323±52 (125-462)
	[0.014]		[0.004]		[NS]	

*mean ± SD; range in parentheses, expressed as per ten thousands of the injected activities in the forearm at T=0; in brackets, level of significance using paired t-test; NS, not significant.

However, among these left-handed volunteers, when AxN activities at T+100 were analyzed with consideration given to the Edinburgh Handedness Inventory, the left-right ratios were as follows: for the pure left-handed participants, 3.60 (413/115), 0.52 (462/874) and 5.11 (220/43); and for the left-handed participants with one ambidextrous-ambilateral component, 0.78 (125/160), 1.10 (386/345) and 0.50 (334/668).

Finally, when the results of the 12 pure right-handed and of the 3 pure left-handed volunteers were pooled, the asymmetry of

the dominant/non-dominant ratio was also statistically significant. The ratios of the AxN activity 100 minutes after injection in the dominant limb versus the AxN activity in the non-dominant limb are higher than 1.30 in ten, between 1.00 and 1.30 in two, and lower than 1.00 in three of the 15 volunteers.

The four participants with one ambidextrous-ambilateral component (three left-handed and one right-handed) had dominant/non-dominant ratios between 1.00 and 1.30 in two cases and lower than 1.00 in two cases.

DISCUSSION

Our results thus show that after intradermal injection of radiolabeled colloids at the level of the forearm, their resorption and/or transport by the lymphatic system (respectively determined by the number of lymphatic termini per cutaneous unit squared and by the number of lymphatic vessels and/or their intrinsic contractile activity) and their accumulation in the axillary nodes capacity are higher in the right upper limbs than in the left upper limbs and more precisely more developed in the dominant upper limbs than in the non-dominant upper limbs. From the anatomical point of view, differences between the lymphatic system of the right and left upper limbs had already been reported. At the "macroscopic" level, larger series have shown that the numbers of axillary nodes (14-16) differ between the right and left upper limbs. At the "microscopic" level, Lubach et al (17) also demonstrated that the lymphatic vessels density (LVD) varies according to the anatomical part of the human body studied. In a more functional approach and at the level of the upper limbs, using fluorescence microlymphography to visualize and study the initial lymphatic network in human dermis, Stanton et al (18) found that none of their three indices of lymphatic density, nor maximum lymphatic spread, differed significantly between the right and left arms (six male and six female subjects combined). Strikingly, we also found no significant difference using an unpaired statistical test when comparing the results of the right injections to the results of the left injections from a group of individuals. In the same paper, Stanton et al noted however that in individual subjects between arm differences were present and that sample size analyses indicated that for men, an examination of 12 subjects would enable the detection of a significant difference in maximum lymphatic density. Our work based on 19 male volunteers confirms their hypothesis. When the right and left forearms of the same person are

compared, the observed axillary lymph node activities following right forearm injections (the results of the extractions of the radiolabeled colloids by the initial lymphatics at the level of the injected sites, of their transport by the lymphatic vessels and of their uptake by the lymph nodes) are significantly higher than lymph node activities following left forearm injections. However, comparing right to left analyzes the effects of lateralization, which is different from handedness. When handedness is taken into account, at least two-thirds of the participants in our study demonstrated a higher activity in the AxNs of their dominant limb (right or left) than in the nodes of their non-dominant limb. More precisely, among the volunteers who said that they were right-handed, the right AxN activity was the same or higher than the left AxN activity in 85% (10 out of 13) of the subjects. On the other hand, among the six who said that they were left-handed, no significant asymmetry was observed. However, the Edinburgh Handedness Inventory identified one ambidextrous-ambilateral component in three of these left-handed volunteers, what may explain the lack of difference in these 6 self-reporting left handed subjects.

At the level of the upper limbs, the highest functionality of the lymphatic system at the level of one limb when compared to the opposite may represent one physiological adaptation to the greatest physical use of the dominant limb compared to the non-dominant one. Stanton et al (19) found in sixteen subjects that LVD was significantly higher at the level of their lower limbs than at the level of their forearm upper limbs. They concluded that at the level of the lower legs, such increase in the initial lymphatic network represents one anatomical adaptation facilitating absorption the higher interstitial fluid loads than the forearms, which are subject to less gravitational loading.

Our observations may have important clinical as well as fundamental implications for scintigraphic investigations of the superficial lymphatic system. These types of

lymphoscintigraphic examinations have two main applications: 1) the demonstration of the lymph nodes draining a tumor-bearing cutaneous area (20,21), and 2) the morphologic and functional evaluation of the lymphatic system (not only of the nodes) in cases of edematous limbs (22-33).

Intradermal injections are ideal for the first application, allowing fast and clear visualization and delineation of the lymphatic vessels and nodes. In clinical practice, intradermal injections are commonly used to demonstrate the sentinel nodes in melanoma-bearing patients (34,35) and in breast cancer cases (36). However, when studying fundamentally the effect of variables on lymphatic imaging, the quantitative data obtained following intradermal injections might need to be reconsidered in light of our results, as should conclusions based on data in which the injection "side" is not precisely described or controlled (37,38).

Regarding the evaluation of limb edemas, there are some reports (31-33) on the use of intradermal injections, but many reports are based on subcutaneous injections. Authors using subcutaneous injections (23-30) report sensitivities usually higher than 90%. In contrast, after intradermal injections, in one study by Nawaz et al in 1992 (32), although combining qualitative and quantitative data, reported a sensitivity of only 66%. The low sensitivity reported by these authors might then be in part explained in light of our results demonstrating normal asymmetries between dominant and non-dominant limbs. Not taking into account this normal asymmetry (the ratio dominant/non dominant is higher than 1.3 in two thirds of our subjects) could have masked the presence of functional and unilateralized underlying pathologies in the Nawaz study using ID injections.

Although demonstrated in the present paper using intradermal injections, such a normal asymmetry might also lead to question some results obtained with subcutaneous injections. Stanton et al found (39) that the removal constant rate (k) of ^{99m}Tc -labeled

hIgG (human IgG) injected subcutaneously in the hands and forearms of women with breast cancer related lymphedema (BCRL) was 25% lower in edematous forearm tissue than in the control arm and that in the non-edematous hand of the BCRL arm, k was 18% higher than in the control hand. In 2006, they also reported (40) that the local lymph drainage rate constant of ^{99m}Tc -labeled hIgG measured over 5 h after SC injection in the subcutis of the ipsilateral swollen hand was $34 \pm 24\%$ less than in the contralateral normal hand ($p=0.013$). More recently, Lane et al (41) reported the results of their paired t-tests run on their data of affected and unaffected arms from breast cancer subjects with (BCRL) and without (BC) lymphedema. Both BCRL and BC had significantly lower axillary node accumulation of their tracer on the affected side at rest ($p=0.014$ and $p=0.019$, respectively). These differences reported by Stanton et al, albeit significant, are low and one might question if they are or not partly related to one simple and normal asymmetry between the studied operated arms and their opposite. On the other hand, one might speculate whether as in Lane's paper, the level of significance might not have been different (higher?) if their analysis had taken into account the lateralization and/or the handedness of their patients. Unfortunately, data in this area are very sparse and incomplete.

To conclude, our work demonstrates the influence of lateralization, and more precisely, of handedness on the results of lymphoscintigraphic investigations at the level of the upper limbs. It confirms the Stanton et al observation (18) that in individual subjects between arm differences are present at the level of the initial lymphatic network in human dermis. Thus, lateralization and handedness represent variables that need to be considered in any study with diagnostic or therapeutic implications (for instance, drug administration) in which the lymphatic system, specifically the upper limb lymphatic system, is involved.

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