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LETTER TO THE EDITOR

Comment to Allegra C, R Sarcinella and M Bartolo, Jr.: Morphologic and functional changes of the microlymphatic network in patients with advancing stages of primary lymphedema. *Lymphology* 35 (2002), 114-120.

Allegra and co-workers have examined patients with primary lymphedema and different degrees of disease severity by the technique of fluorescence microlymphography, a topic that merits attention. However, the article contains certain ambiguities, inaccuracies, and omission of specific relevant literature from the citation list.

We disagree with the first sentence. Allegra states that "Microlymphography was first performed using dextran 40,000 but its relatively small molecular size yielded confusing lymphatic transport data as the marker was partially absorbed by blood capillaries and excreted by the kidneys". Fluorescence microlymphography was introduced in 1981 by Bollinger and co-workers in *Circulation* (1). In that paper it was stated: "The contrast was enhanced by the use of FITC-dextran 150,000 instead of FITC-dextran 40,000." Accordingly, dextran of molecular weight 150,000 kDa or even larger molecules were used in a series of papers from the Zurich group (e.g. 2-8), from Mortimer's group in London (9-12), and Leu in Boston and Zurich (13,14). We have compared the use of FITC-dextran 40,000 and 150,000 and showed that in normals the large molecules do not leave the microlymphatics and must be cleared by the lymphatic system, whereas the smaller molecules may pass the blood endothelial barrier (15). We do not agree that there is "confusion" about the extensive published data.

Most of Allegra's findings are not new. The demonstration of a larger area of dye-filled microlymphatic network in primary lymphedema compared with normals was described in our first paper (1) and, subsequently, publications 2-4,6,12 that are not cited. Their increased lymphatic capillary diameters are not discussed in relation to our earlier observations published in *Lymphology* (16,17). Similarly, microlymphatic pressure data obtained using the servo-nulling technique are quoted without reference to earlier measurement in healthy controls (5), although the contribution of Intaglietta in the development of this technique is noted. Moreover, the Zurich group previously observed microlymphatic hypertension in primary lymphedema (6, 7).

Other concerns include the following. The types of lymphedema examined are not adequately defined. Was there distal hypoplasia? Was there associated venous disease? Were the control subjects historical or case-controlled? The Methods section is missing details. Concerning measurement of capillary diameter, "morphometric computerized elaboration" is not informative. Lymphatic capillary diameter can be a difficult measurement to perform and the measurement technique used can substantially affect the results. Given the low number of controls, the magnitude of the coefficients of variation apparent from *Table 2*, and the use of unpaired statistical analysis, it is surprising that the "differences" between controls and Groups I-III reached significance at a level of 0.001. Initial lymphatics are presented as a dimensionless number; the vessel needs to be defined. Flow velocity,

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which was also measured by the Zurich group (8), is expressed in seconds when the usual measurement given is length (µm) per second. A specific statement regarding hospital ethics committee approval of the project is not provided.

These shortcomings limit the value of this paper in advancing understanding of primary lymphedema.

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Reply:

In the first part of the text we briefly outlined the history of microlymphography and explained why scientists abandoned dextran 40,000 for dextran 150,000.

The word "misleading" is indeed preferable to "confusing" in the second paragraph of the foregoing Letter. Moreover, since we intended to provide only a historical introduction to the topic, we did not deem it appropriate to go into excessive detail.

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Regarding the findings reported in our paper, the authors of the research did not claim to be the first, but only attempted to provide a contribution to the understanding of the topic. Publishing data about recurrent varicose veins should not be precluded because many authors have already written about them.

As for the servo-nulling technique, which is currently used routinely to perform micro-lymphography, we considered it sufficient to cite the scientist who devised the instrument.

The diagnosis of primary lymphedema was formulated after a clinical-retrospective evaluation. Patients with venous diseases were excluded as in the research studies by other authors.

Patients did not undergo traditional lymphography. Hypoplasia is quite rare and was not a subject of our study. The staging of primary lymphedema was by "compressible, moderately compressible, noncompressible," according to Földi's classification.

Control subjects were not historical.

Measurement of capillary diameter utilized Capiflow software 3.2, according to manufacturer's instructions and properly calibrating the set-up in the "calibration menu" according to the magnification. When using a 3.2x lens and a x5 zoom, a value of 3.7 was assigned to the calibration menu. We did not specify this point because it is routine for microlymphography.

Data collected from healthy controls confirm the results of previous investigations carried out by myself and other authors.

The test (Student's t test) used was not specified, but is the standard analysis for studies on small samples such as an observation number of lower than 30. The formula applied was:

$$t = \frac{X-\mu_0}{\frac{S}{\sqrt{n}}}$$

where: X = mean of the sample; μ_0 = mean of the control group; S = unbiased estimator of the standard deviation; n = number of observations of the sample.

In *Table 2*, the elements necessary to calculate Student's t test were reported. Accordingly, the significance reported in the paper can be confirmed as accurate for the mean±SD number of initial lymphatics visualized per field in the subjects in each group.

Velocity of fluorescence staining is well-defined on page 116 and is represented by the time necessary to visualize the lymphatic network after the injection of the vital dye, a simple measurement easily visualized on the monitor.

My Department of Angiology encompasses a Microcirculation Laboratory. Patients are studied in this Laboratory to evaluate the results of pharmacological therapy or physiotherapy or to investigate the lymphatic microhemodynamic differences resulting from lymphatic vessel microsurgery. Review by the Hospital Ethical Committee has not been required for these clinically indicated studies although informed consent of the patients is obtained.

I apologize for insufficiently citing the school of Professor Bollinger, with whom I have long been friendly and to whom I am grateful for teaching microlymphography to two of my co-workers, Dr. Bartolo and Dr. Cassiani, 15 years ago. I am pleased, however, that the critical Letter is being published, as I too have the opportunity to respond and clarify the many points raised by the Letter to *Lymphology*. The exchange will surely expand the readers' understanding of the issue.

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