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Increased Microvascular Filtration and Vascular Endothelial Growth Factor-D associated with Changed Lymphatic Vessel Morphology in Breast Cancer Treated Patients

Andreas L. Johannessen, MD*‡‖
Mathias Alstrup, MD*‡
Vibeke E. Hjortdal, MD, PhD, DMSc‡§
Johan Palmfeldt, MSc, PhD‡¶
Birgitte V. Offersen, MD, PhD†‡
Sheyanth Mohanakumar, MD PhD‡**††

Background: Vascular endothelial growth factors (VEGF) and inflammatory cytokines are indicated to be implicated in lymphedema development. We aimed to describe changes in microvascular filtration and VEGFs in a patient cohort vulnerable to breast cancer–related lymphedema development correlated with data on lymphatic morphology and function.
Methods: Consecutive node-positive breast cancer patients operated in the axilla and evaluated approximately 12 months after adjuvant locoregional radiotherapy were studied. Capillary filtration rate (CFR) and isovolumetric pressure of the arms were measured by strain gauge plethysmography, and 13 blood proteins were quantified by Luminex and Elisa technology in 28 patients and 18 healthy controls.
Results: The CFR was reduced in both arms from baseline to 1-year follow-up (ipsilateral: $P = 0.016$ and contralateral: $P = 0.001$). When stratifying lymphatic complications (morphologic abnormalities and/or breast cancer–related lymphedema), CFR reached a lower steady-state in the arms with normal morphology (I:$P = 0.013$ and C:$P = 0.013$) whereas the ipsilateral arm with lymphatic complications remained unchanged ($P = 0.457$). In patients with lymphatic abnormal vessels, the levels of VEGF-D were 86% higher than in patients with normal lymphatic vessels ($P = 0.042$), whereas levels of VEGFR-3 were 64% higher ($P = 0.016$).
Conclusions: Through one year of follow-up, CFR did not decrease in the lymphatic complicated treated arms as observed in noncomplicated treated arms. The patients had increased levels of VEGF-D and VEGFR-3. This correlation suggests that VEGF plays a role in the appearance of subcutaneous abnormal lymphatic vessels in the treated arms, which also maintain a fluid filtration/drainage mismatch up to one year after breast cancer treatment. *(Plast Reconstr Surg Glob Open 2024; 12:e5968; doi: 10.1097/GOX.0000000000005968; Published online 19 July 2024.)*

*From the *Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital, Aarhus, Denmark; †Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark; ‡Department of Clinical Medicine, Aarhus University, Aarhus, Denmark; §Department of Cardiothoracic Surgery, Rigshospitalet, Copenhagen, Denmark; ¶Research Unit for Molecular Medicine Research, Aarhus University Hospital, Aarhus, Denmark; ‖Department of Vascular Surgery, Hospitalsenheden Midt, Viborg, Denmark; **Department of Radiology, Aarhus University Hospital, Aarhus, Denmark; and ††Department of Radiology, Regionshospitalet Horsens, Horsens, Denmark.*

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INTRODUCTION

Breast cancer related lymphedema (BCRL) is a feared complication amongst patients treated for breast cancer. Despite great efforts trying to avoid lymphedema in the arm during the months to years after being cancer free, regrettably, up to 20% of the patients develop a degree of lymphedema, and the only treatment is conservative congestion therapy.[1]

Animal studies propose that lymphedema development is subject to an inflammatory process with formation of fibrotic tissue in progressed disease.[2–5] In mice models, CD4+ T cell signaling has been demonstrated to be crucial for maintaining this process. Vascular endothelial growth factors C and D (VEGF-C/D) have been highlighted as key molecules in the process of lymphangiogenesis and lymphedema development, but it remains to be established

Disclosure statements are at the end of this article, following the correspondence information.

whether VEGF-C/D effects are beneficial or detrimental.[6–19] VEGF-C participates in the process of angiogenesis and lymphangiogenesis responsible for increasing both blood and lymphatic endothelial permeability as well as the diameter of the lymphatic vessels.[9–11] Further, this protein induces growth, sprouting, and remodeling of lymphatic vessels in vivo, and excess VEGF-C concentrations lead to hyperplasia of the lymphatics in the dermis.[12] VEGF-D is structurally and, thus, functionally related to VEGF-C, and together they form a subfamily within VEGFs, being able to bind to VEGFR-3, a receptor predominantly expressed on endothelial cells of lymphatic vessels.[11]

Recently, we have demonstrated that in a cohort of node-positive breast cancer patients, the lymphatic function in the ipsilateral arm decreased after 1 year of follow-up posttreatment. We found that 46% of patients presented changed lymphatic vessel morphology associated with reduced capacity of lymphatic vessel contraction.[13,14]

We aimed to describe the change in the upper extremity microfiltration through one year of follow-up in the exact same patient cohort in which we previously reported functional and morphological data. Therefore, this present study focused on plethysmography data and blood samples, including levels of VEGFs and inflammatory cytokines at the follow-up examination, interpreted in relation to the functional and morphological results previously published.[13]

Takeaways

Question: How do capillary microfiltration and plasma levels of vascular endothelial growth factors change in the long term after adjuvant breast cancer therapy?

Findings: We investigated the capillary filtration rate (CFR) and levels of VEGFs in 29 breast cancer patients with 1-year follow-up. CFR reached a lower steady-state after 1-year follow-up overall, but CFR in the treated arm with lymphatic complications remained unchanged. Levels of VEGF-D and VEGFR-3 were elevated in patients with lymphatic complications.

Meaning: Changed lymphatic vessel morphology may be associated with a fluid filtration/drainage mismatch while VEGFs may play a role in the appearance of subcutaneous spiderwebs of abnormal lymphatic vessels.

MATERIAL AND METHODS

Ethical Approval

The Regional Committee on Health Research Ethics of the Central Denmark Region (1-10-72-193-18) has approved this study. The study is registered on ClinicalTrials.gov (identifier: NCT03572998). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, revised in 2013, and all participants provided written informed consent before enrollment. This study meets the STROBE guidelines.

Study Design and Population

This study focused on plethysmography and blood sample data collected in a study already published with lymphatic functional and morphological data.[13] The study was a prospective cohort study, set up as a follow-up on a previously examined cohort from September 2018 to December 2019 at Aarhus University Hospital by Alstrup et al,[14] reporting the baseline examinations. We examined the same cohort of patients during October 2019 to June 2020, which was between 6 and 12 months after completion of the primary lymphatic examination and approximately 1 year after ending radiotherapy, which we refer to as the follow-up examination. Therefore, in this study, we report data of CFRs from the baseline examination compared with our capillary filtration rate (CFR) findings.

The population consisted of 32 women with unilateral breast cancer who all completed surgery, systemic therapy (chemotherapy and/or endocrine therapy), and locoregional radiotherapy less than six months before the primary lymphatic examination. Surgical procedure consisted of either lumpectomy or mastectomy including either sentinel node biopsy or axillary lymph node dissection. All patients participated in the Danish Breast Cancer Group RT Skagen trial 1 (NCT02384733). For detailed information about patients' adjuvant radio-, chemo- and endocrine therapy as well as patient recruitment and exclusion criteria, we refer to the primary study.

Patients included in the follow-up study were followed up regarding potential development of BCRL. They were contacted on September 1, 2020 by phone; on January 18, 2021; and finally, on July 6, 2021. BCRL status was validated through medical records. If clinical BCRL had developed before the primary examination, the patient was excluded, while subsequent BCRL development before or after the follow-up examination was registered. Therefore, BCRL patients consisted of a group of patients that at the time of follow-up examination already had or later were at risk of BCRL.

The arm adjacent to the treated breast was labeled "ipsilateral," whereas the nontreated side was labeled "contralateral," enabling patients to serve as their own control.

In this study, we defined BCRL as clinically evident lymphedema in the arm or hand diagnosed and described in the electronic medical records by experts at the lymphedema clinic, Aarhus University Hospital. We used the lymphedema criteria defined in the Danish Breast Cancer Group RT Skagen trial 1. The definition of arm lymphedema was greater than or equal to 10% increased arm circumference measured 15 cm proximal and/or 10 cm distal of the olecranon on the ipsilateral arm compared with the contralateral arm. If the patient used an arm sleeve, she was asked to not wear this 24 hours before measurement. Measurements were supplemented by patient-reported outcome measures with questions of subjective sensations like heaviness and numbness of the arm. This definition is in harmony with the After Mapping of the Axilla: Radiotherapy or Surgery (AMAROS) trial.[17]

ENDPOINTS

The primary endpoint of this study was the CFR measured by plethysmography, whereas the blood sample data were secondary endpoints.

Table 1. Characteristics of Participants Who Completed Breast Cancer Treatment

	Breast Cancer–Treated Patients (n = 29)	BCRL Patients (n = 6)	Non-BCRL Patients (n = 23)	*P*
Demographics				
Age, y	55 ± 11	47 ± 7	58 ± 11	0.042
Weight, kg	75 ± 15	71 ± 20	76 ± 14	0.524
Height, cm	167 ± 6	161 ± 5	168 ± 6	0.010
Body mass index, kg/m^2	27 ± 5	28 ± 8	27 ± 4	0.706
Currently smoking, n (%)	4 (14)	1 (17)	3 (13)	0.627
Axillary surgical type, n (%)				0.057
Sentinel node	10 (34)	0 (0)	10 (43)	
ALND	19 (66)	6 (100)	13 (57)	
Lymph node removed	13 ± 9	14 ± 6	12 ± 9	0.579
Surgery, n (%)				0.457
Mastectomy	8 (28)	1 (17)	7 (30)	
Lumpectomy	21 (72)	5 (83)	16 (70)	
Chemotherapy, n (%)	21 (72)	4 (67)	17 (74)	0.543
Endocrine therapy, n (%)	26 (90)	4 (67)	23 (96)	0.100
Radiation treatment n (%)				0.487
50Gy/25 fractions	12 (41)	3 (50)	9 (61)	
40Gy/15 fractions	17 (59)	3 (50)	14 (39)	
Time since treatment, d				
Primary examination	35 ± 23	30 ± 18	36 ± 25	0.572
Follow-up examination	313 ± 65	328 ± 70	309 ± 65	0.516
Total follow-up time since treatment, d	789 ± 112	820 ± 99	781 ± 116	0.452

Data reported as means ± SDs or absolute numbers and percentages of patients.
P values between BCRL and non-BCRL patients.
ALND, axillary lymph node dissection.
Adapted with permission from Johannessen AL, Alstrup M, Hjortdal VE, et al. Lymphatic function decreases over time in the arms of breast cancer patients following treatment. *Plast Reconstr Surg Glob Open*. 2022;10:e4507.

STUDY PROCEDURE

Capillary Filtration Rate

The CFR (ml × 100 ml^{-1} tissue × min^{-1}) of the arms was measured using a strain gauge plethysmography setup (Hokanson EC6 and Hokanson E20; Marcom Medical, Denmark) connected to a PC using an analog-to-digital converter (ADInstruments, Oxford, United Kingdom) and analyzed using LabChart 7 software. Initially, a sphygmomanometer cuff was placed around the brachium of the participant. The cuff was inflated to a pressure of 20 mm Hg and increased with 10 mm Hg every 3 minutes until a pressure of 80 mm Hg was reached. The change in circumference was recorded continuously by the strain gauge, placed distally to the cuff. Initially, the increase in venous pressure resulted in a rapid, nonlinear increase in the volume of the arm, due to venous distension. Subsequently, the greater hydrostatic pressure in the capillaries increased the CFR, resulting in a modest linear increase in the circumference of the arm, due to an increase in the filtration and, thus, interstitial fluid volume.

Blood Sampling

At the end of the examination, venous blood was collected from a cubital vein by medical laboratory assistants in 10 mL EDTA tubes and 10 mL serum tubes. Plasma was immediately stored on ice, and the following procedures were performed with cooling techniques. Samples were separated by centrifugation at 1800 g for 10 minutes and immediately thereafter, frozen in 1.8 mL cryotubes at minus 80 degrees until analysis.

The control group consisted of 18 healthy women between the age of 34 and 85 years. Exclusion criterion was previous or current cancer. Blood samples were collected with the same technique as for patients.

The protein concentrations of a panel of 11 selected proteins (VEGF-D, VEGFR-3, hepatocyte growth factor, IFN-γ, IL1-β, IL-2, IL-4, IL-6, IL-8, IL-10, and IL-13) were simultaneously quantitated by the bead-, antibody-, and fluorescence-based Luminex technology on Magpix instrumentation (Millipore Corp), using an 11-plex Luminex kit from Thermo Fisher Scientific. In a separate assay, VEGF-C was quantitated by ELISA (R&D systems) and endostatin in a single-plex Luminex assay (Procarta, Thermo Fisher Scientific). The plasma samples (25 µL) were processed, in two independent duplicates, according to instructions of the kit manufacturer. The absolute protein concentrations were obtained by a seven-point calibration curve, using five-parameter logistic curve-fit, with known standards ranging over more than three orders of magnitude.

Data Analysis and Statistics

Results were reported as means of concentrations ± SD for continuous data and binary data as absolute numbers. In Table 2, the number of participants (n) were reported within brackets after SD, because it varied between groups and measurements due to exclusion of samples under detection limit.

Using Stata/SE 15.1 for Mac (StataCorp, Tex.), data were tested for significance in difference between groups with a paired and unpaired Student *t* test as well as two-way

Table 2. Plasma Levels of Selected Blood Proteins in Patients and Control Group

Blood Protein	Patients					Control Group
	Joint Group N = 28	BCRL N = 6	Non-BCRL N = 22	w/ Abnormal N = 14	w/o Abnormal N = 14	N = 18
VEGF-C, pg/ml	232 ± 115	219 ± 68	235 ± 126	203 ± 91	260 ± 132	181 ± 75
VEGF-D, pg/ml (n)	21 ± 15 (27)	17 ± 9	21 ± 16 (21)	26 ± 19*	14 ± 5 (13)	17 ± 14
VEGFR-3 pg/ml (n)	22951 ± 13056	26629 ± 22449	21947 ± 9709	28541 ± 8594*	17361 ± 8594	18584 ± 11075 (17)
Endostatin, pg/ml (n)	79 ± 43	84 ± 40	78 ± 44	76 ± 42	83 ± 44	91 ± 51

Data reported as means ± SDs. The number of participants (n) are reported within brackets after SD.
VEGFR-3, Vascular Endothelial Growth Factor Receptor 3
W/abnormal, with subcutaneous morphological lymphatic vessel abnormalities.
W/o abnormal, without subcutaneous morphological lymphatic vessel abnormalities.
*Significant ($P < 0.05$) difference between patients with or without lymphatic abnormalities.
The labels of the groups "w/ abnormal" and "w/o abnormal" relates to whether subcutaneous morphological lymphatic vessel abnormalities in the ipsilateral arm were observed.

Table 3. Detection/No Detection

Blood Protein	Patients (N = 28)		Control (N = 18)		Detection Limit	Fisher Exact Test
	Detection	No Detection	Detection	No Detection	(pg/mL)	(*P*)
HGF, n	6	22	3	15	7.28	0.501
IFN-γ, n	0	28	0	18	17.92	
IL-1β, n	7	21	2	16	2.56	0.221
IL-2, n	15	13	5	13	8.18	0.077
IL-4, n	0	28	0	18	15.09	
IL-6, n	0	28	0	18	10.55	
IL-8, n	0	28	0	18	2.19	
IL-10, n	4	24	3	15	2.30	0.570
IL-13, n	0	28	0	18	3.03	

Data reported as absolute numbers.
IFN-γ, Interferon gamma; IL, Interleukin.

analysis of variance (ANOVA) for normal distributed data. Fisher exact test was used for binary data. Significance level was set to 0.05 in all tests. CFR was calculated by extracting the filtration (mL/100 mL/minute) from each occlusion pressure and compared between study dates and arms by two-way ANOVA.

Isovolumetric pressure (mmHg) was calculated from the intercept of capillary filtration coefficient (ml 100^{-1} tissue $minute^{-1}$ mm Hg^{-1}) and the *x*-axis. Because the remaining nine proteins had concentrations under the detection limit or missing data (N/A), they are presented separately in Table 3 as absolute numbers to whether the concentration was detectable or not.

RESULTS

Twenty-nine female patients completed the follow-up examination. From one patient, it was not possible to withdraw a blood sample; thus, blood samples from 28 patients were analyzed.

Strain gauge plethysmography was completed on both visits by 21 participants. Data from three participants were excluded due to artifacts hindering analysis, leaving a final count of 18 participants for analysis.

Mean total follow-up time was 789 ± 112 days. Patient demographics are summarized in Table 1. Figure 1 shows an example of normal and abnormal lymphatic morphology. (Fig. 1)

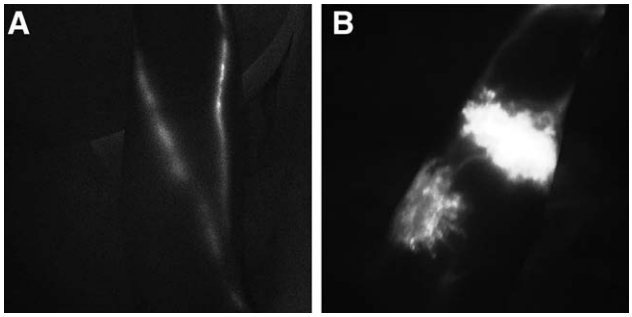

Fig. 1. Lymphatic morphology. A, Linear pattern with fairly straight, distinguishable vessels, which is considered a normal pattern. B, Illustration of two areas in the forearm with dermal lymphatic rerouting of tiny lymphatic vessels assembling small "spiderwebs," which are considered abnormal lymphatic morphology.

Blood Samples

Table 2 shows the concentrations of the four quantifiable proteins (VEGF-C, VEGF-D, VEGFR-3, and endostatin). We detected a tendency for elevated VEGF-C in patients compared with controls ($P = 0.104$). Levels of VEGF-D and VEGFR-3 were significantly higher in patients with lymphatic abnormalities compared with normal lymphatic vessel morphology. The remaining nine blood proteins, hepatocyte growth factor, interferon gamma (IFN-γ), and seven interleukins, had concentrations close

to detection level, concentrations under detection limit, or missing data (N/A), and are therefore presented separately in Table 3 as absolute numbers to whether the concentration was detectable or not. Only IL-2 showed a tendency of differential detectability in patients compared with the healthy controls ($P = 0.077$; Table 3).

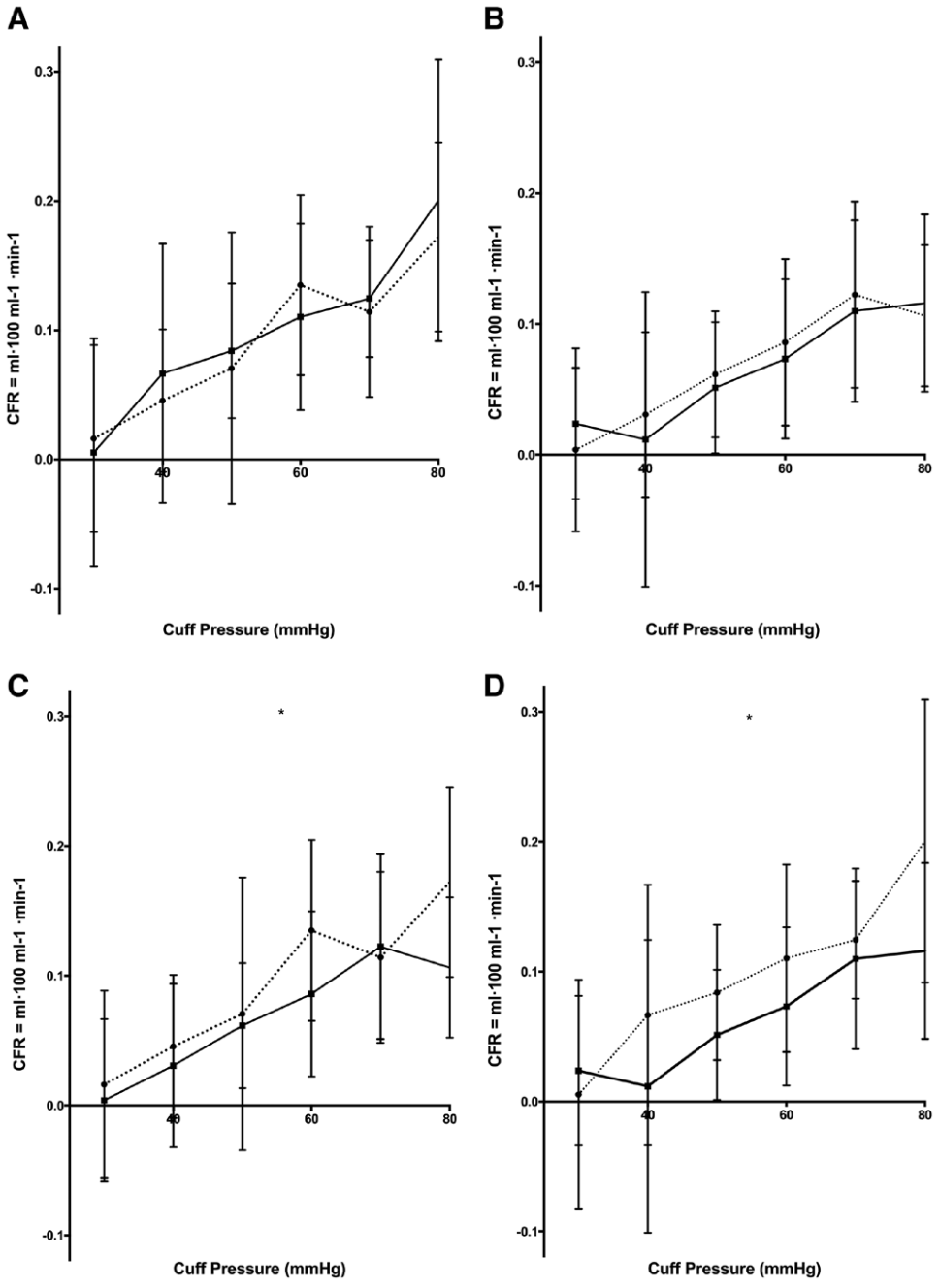

Fig. 2. Illustration of the CFR at increasing pressure steps in both the ipsi- and contralateral arms in different comparisons. Data were reported as means with SD. (*) indicates $P < 0.05$. N = 18. The isovolumetric pressure from where extravasation and lymphatic drainage begins to be equal or higher were between 30 mm Hg and 80 mm Hg for all measurements. A, No significant difference between primary ipsilateral (●) compared with the primary contralateral (■) (two-way ANOVA, $P = 0.548$). B, No significant difference between follow-up ipsilateral (●) compared with the follow-up contralateral (■) (two-way ANOVA, $P = 0.615$). C, CFR at follow-up for the ipsilateral arm (■) was lower compared with the primary examination of the ipsilateral arm (●) (two-way ANOVA, $P = 0.016$). D, CFR at follow-up for the contralateral arm (■) was lower compared with the primary examination of the contralateral arm (●) (two-way ANOVA, $P = 0.001$).

Capillary Filtration Rate

Figure 2 demonstrates CFRs during strain gauge plethysmography. At follow-up, there was no difference between the ipsilateral and contralateral arms.

Over time, CFR decreased from the primary to the follow-up examination for both arms (Fig. 2C, D; n = 18; two-way ANOVA: ipsilateral arm $P = 0.016$, contralateral arm $P = 0.001$).

At follow-up, when stratifying lymphatic complications (morphologic abnormalities and/or BCRL versus normal morphology) CFR reached a lower steady-state in the arms with normal morphology (Fig. 3C, D;

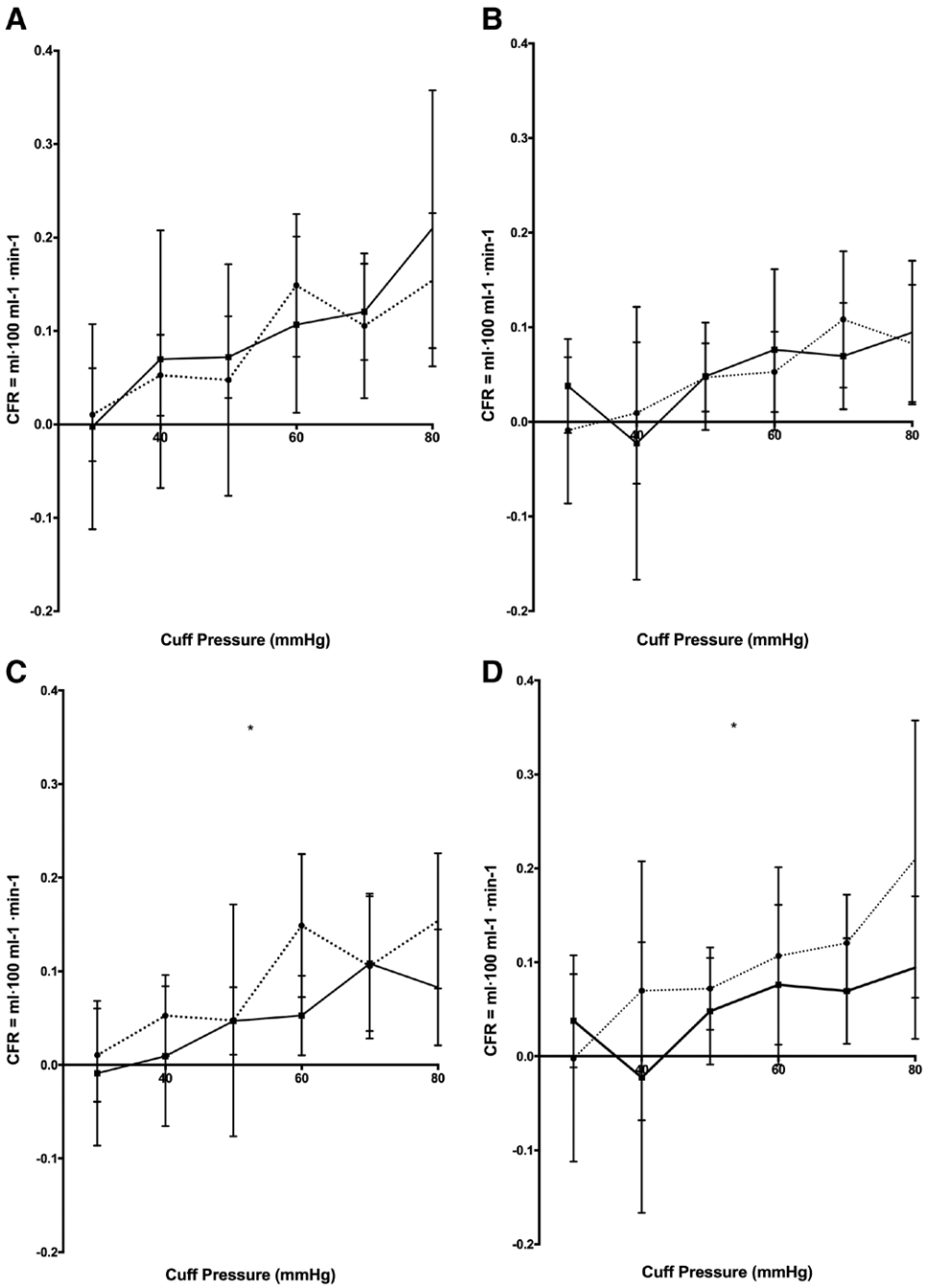

Fig. 3. Illustration of the CFR at increasing pressure steps in both the ipsi- and contralateral arms for patients without lymphatic complications. Data were reported as means with SD. (*) indicates $P < 0.05$. N = 9. A, No significant difference between primary ipsilateral (●) compared with the primary contralateral (■) (two-way ANOVA, $P = 0.580$). B, No significant difference between follow-up ipsilateral (●) compared with the follow-up contralateral (■) (two-way ANOVA, $P = 0.878$). C, CFR at follow-up for the ipsilateral arm (■) was lower compared with the primary examination of the ipsilateral arm (●) (two-way ANOVA, $P = 0.013$*). D, CFR at follow-up for the contralateral arm (■) was lower compared with the primary examination of the contralateral arm (●) (two-way ANOVA, $P = 0.013$*).

I:P = 0.013 and C:P = 0.013) and in the contralateral arms in patients with lymphatic complications (Fig. 4D, P = 0.019), whereas the ipsilateral arm with lymphatic complications remained unchanged (Fig. 4C, P = 0.457).

The isovolumetric pressure (the pressure point with balance between fluid extravasation and lymphatic drainage) was similar for both arms between the primary and follow-up examinations (Fig. 5, t test, P = 0.219, n = 18).

DISCUSSION

In this study, we showed quantifiable evidence of microcirculatory changes in early breast cancer patients

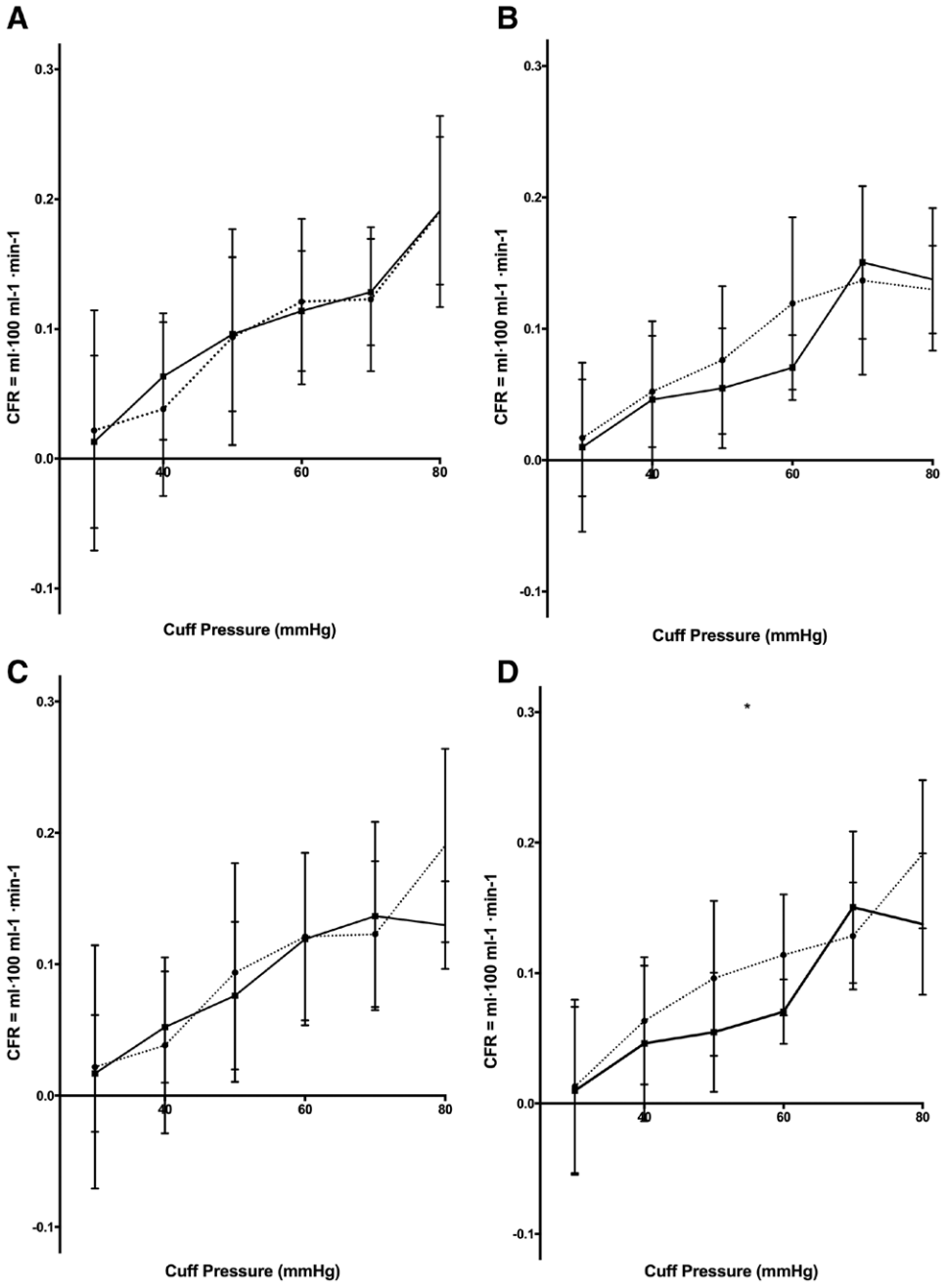

Fig. 4. Illustration of the CFR at increasing pressure steps in both the ipsi- and contralateral arms for patients with lymphatic complications. Data were reported as means with SD. (*) indicates $P < 0.05$. N = 9. A, No significant difference between primary ipsilateral (●) compared with the primary contralateral (■) (two-way ANOVA, P = 0.815). B, No significant difference between follow-up ipsilateral (●) compared with the follow-up contralateral (■) (two-way ANOVA, P = 0.311). C, No significant difference between CFR at follow-up for the ipsilateral arm (■) compared with the primary examination of the ipsilateral arm (●) (two-way ANOVA, P = 0.457). D, CFR at follow-up for the contralateral arm (■) was lower compared with the primary examination of the contralateral arm (●) (two-way ANOVA, P = 0.019*).

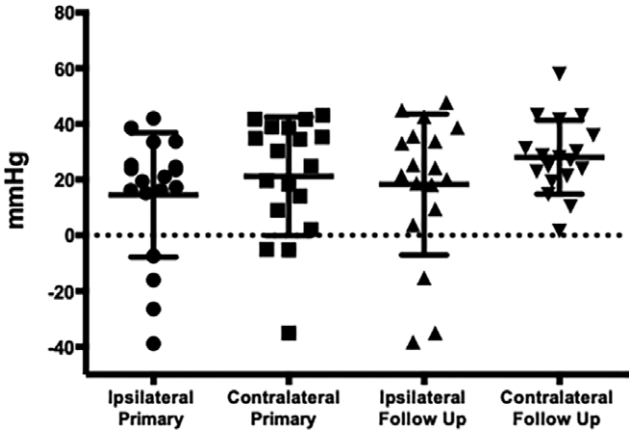

Fig. 5. Isovolumetric pressures during primary and follow-up examination for both arms, n = 18.The isovolumetric pressures represent the highest pressure where extravasation and lymphatic drainage are equal. No significant difference between primary ipsilateral (●) compared with the primary contralateral (■) (paired Student *t* test, *P* = 0.379), follow-up ipsilateral (▲) compared with the follow-up contralateral (▼) (paired Student *t* test, *P* = 0.142), primary ipsilateral (●) compared with follow-up ipsilateral (▲) (paired Student *t* test, *P* = 0.591) and primary contralateral (■) compared with follow-up contralateral (▼) (paired Student *t* test, *P* = 0.254).

one year after ended adjuvant radiotherapy. Declining CFRs in both arms from the primary examination to follow-up were detected, but interestingly, we discovered that the least decline of CFRs was in the treated arm in a combined group of patients with lymphatic complications. Further, the same group of patients was found to have elevated plasma levels of VEGF-D and VEGFR-3.

This is the first longitudinal study in human demonstrating that the development of lymphatic morphological changes have corresponding changes in CFR and increased levels of vascular endothelial growth factors.

Capillary Filtration

Lymphatic reabsorption is critical in maintaining the tissue fluid balance, while venous reabsorption is much less important.[16–18] Thus, a provoked mismatch between capillary filtration and lymphatic drainage could cause tissue fluid accumulation. In the situation where the lymphatic drainage may be compromised, such as in the arm of a breast cancer treated patient, regulatory mechanisms are required to reestablish an acceptable fluid balance.

In this study, we demonstrated an overall CFR decrease in both arms between the primary and follow-up examination, suggesting an initial systemic microcirculatory response after breast cancer treatment. In patients with lymphatic complications, the CFR did not decrease to the same extent. This suggests that these patients sustain an increased interstitial fluid load, presumably with a greater demand on the lymphatic drainage system in accordance with Jensen et al.[19]

Based on our results, the association between high CFRs and lymphatic complications is strengthened. We consider that fluid accumulation in the interstitial compartments could partly be caused by the continuously elevated extravasation of fluid aggravated by the formation of new non- or less-functional lymphatic vessels.

Vascular Endothelial Growth Factors

In our previous study focusing on lymphatic function and morphology, we found that 46% of patients at follow-up had changed lymphatic morphology with a degree of lymphatic rerouting, visualized as subcutaneous spiderwebs of unorganized tiny lymphatic vessels.[13] We now report that this subgroup (n = 14) had increased plasma levels of VEGF-D and VEGFR-3 compared with patients with normal linear lymphatic morphology (n = 14), supporting the hypothesis that the lymphatics of these patients were challenged and thus signaling for growth and remodeling. We are not aware of any studies investigating normal or abnormal serum levels of these molecules in a post breast cancer cohort. Compared with our own control group of healthy women, the absolute concentrations were not dramatically elevated; however, significant differences within the patient cohort stratified on lymphatic vessel morphology may still be biologically relevant in the explanation of lymphedema susceptibility. Itai and colleagues showed three-dimensional morphological changes of lymphatic capillaries with increasing severity of lymphedema. In early stage lymphedema, loss of button-like loose intercellular adhesion, facilitating proper drainage, was linked to VEGF-A and -C secreting macrophages in humans.[20] Mice models have demonstrated that lymphatic obstruction induces lymphangiogenesis via the VEGF-C/VEGFR-3 pathway, generating immature and leaky lymphatic vessels that are essential in later lymphedema development. Early-stage lymphedema has been associated with significant increased lymphatic branching caused by VEGF-C production.[3,6] Further, increased VEGF-C expression in mice after lymphatic injury demonstrated exacerbated lymphedema, while inhibiting VEGF-C/D reduced lymphedema development and impaired lymphangiogenesis.[9] This molecular model would explain the pattern of the unorganized network of neolymphatics we visualized in the arms of our patients and furthermore suggests that immature lymphatics or lymphatics with changed junctional identity were not effectively contributing to the overall lymphatic function.

Another important key feature of VEGFs in general is the ability to increase microvascular permeability. Thus, increased VEGF-D levels may have been responsible for the elevated CFRs demonstrated in the same subgroup of patients with lymphatic morphological changes, as discussed above. Opposite, the patient group with normal lymphatic vasculature did not demonstrate increased VEGF-D as well as decreasing CFR significantly during 1 year of follow-up. From this, it follows that VEGF-D may play a detrimental role in lymphedema development. Maintaining high levels of VEGF-D may stimulate to continuously elevated microfiltration and further signaling for a nonfunctional lymphangiogenesis.

Interleukins

In animal models, it is well established that lymphedema development is driven by different inflammatory

mechanisms.[4] Several independent studies in mice models have suggested a key role of CD4+ T cells with CD4+ signaling crucial for lymphedema development. Inhibiting CD4+ cells prevented initiation and progression of lymphedema by reducing tissue fibrosis and improving lymphatic function.[2,3] Therefore, it was interesting that we did not detect any appreciable cytokine activity, or IL-4 and IL-13, which are CD4+ signaling cytokines. Only IL-2 showed tendency toward a significant difference in detectability, but whether this indicates IL-2 activity in the patients remains uncertain. Similar negative findings of cytokine activity in BCRL patients were shown by Jensen et al.[21] Lymphedema is a chronic degenerative process that can take months to years to develop. We consider that the underlying pathogenic course might have different steps of inflammatory activity. Knowing that interleukins often are regulated in an on/off fashion, it seems possible that the course of inflammation progresses in steps with various periods of inflammatory inactivity in between. This can also explain why the time course of lymphedema development is so different amongst patients.

Study Limitations

The strain gauge plethysmography elastic band was extremely sensitive, so that small movements or deep breaths could influence the recorded sequence. Several patients could not comply with these requirements, consequentially being excluded from analysis. However, the inability to comply with this exercise was expected to be independent of lymphatic function and blood protein levels.

Blood samples were collected once at the follow-up examination approximately 1 year after ending radiotherapy, but unfortunately, blood samples were not collected at the primary examination. The absence of longitudinal blood protein levels is a significant limitation. A normal range of VEGF proteins has, to our knowledge, not been described previously, and comparisons with research literature may be complicated by different blood sample processing techniques, length of blood sample storage, and method of analysis. However, our intracohort comparison is expected to be independent of these factors. Given the pronounced temporal variation of lymphedema development, more frequent blood sampling might increase the chance of elucidating inflammatory cytokine activity.

CONCLUSIONS

In this longitudinally prospective study of a consecutive cohort of node-positive early breast cancer patients following surgery and locoregional radiotherapy, we are the first to demonstrate changes in the microfiltration through 1 year of follow-up, comparing with blood samples of vascular endothelial growth factors and inflammatory cytokines and correlated to subcutaneous alterations in lymphatic vessel morphology and lymphedema status.

Through 1 year of follow-up, we demonstrated that CFRs were not decreasing in patients with lymphatic complications, as observed in noncomplicated groups, and they also had increased levels of VEGF-D and VEGFR-3. With these correlations, this study suggests that lymphatic complications might be associated with a fluid filtration/drainage mismatch and supports a possible explanation of the appearance of subcutaneous spiderwebs of abnormal lymphatic vessels. Further, it provides evidence that axillary surgery and RT affect both the blood vessels and the lymphatic vessels distal from the lymphatic injury up to one year after breast cancer treatment. The exact mechanisms need further investigation.

Andreas L. Johannessen, MD
Department of Cardiothoracic and Vascular Surgery
Aarhus University Hospital
Palle Juul-Jensens Boulevard 99
DK-8200 Aarhus N, Denmark
E-mail: andrjh@rm.dk

DISCLOSURES

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