Table 5 HR of regression from current smoking CIN1/2 according to the serum micronutrients and nutrient intake questionnaire

	n	Person-months	Events	Cumulative 2-year rate (95 % CI)	Hazard ratio for regression (95 % CI)			
					Unadjusted	*p* value	Adjusted model	*p* value
Serum retinol							*p* for trend	0.43
Low (<55.2)	47	614.0	27	64.0 (49.2–78.6)	1		1	
Medium (55.2–67.9)	38	417.6	24	70.5 (53.4–85.7)	1.29 (0.74–2.23)	0.369	1.54 (0.87–2.76)	0.141
High (>67.9)	57	780.5	21	42.9 (30.1–58.3)	0.60 (0.34–1.06)	0.08	0.54 (0.29–1.00)	0.05
Serum α-carotene							*p* for trend	0.898
Low (<5.1)	59	751.9	33	62.5 (49.2–75.8)	1.00		1.00	
Medium (5.1–9.7)	53	689.6	22	49.9 (35.3–66.7)	0.72 (0.42–1.24)	0.24	0.85 (0.48–1.53)	0.595
High (>9.7)	30	370.6	17	61.8 (43.6–80.2)	1.04 (0.58–1.87)	0.886	1.23 (0.63–2.39)	0.537
Serum β-carotene							*p* for trend	0.667
Low (<28.3)	63	788.0	31	58.1 (44.6–72.2)	1.00		1.00	
Medium (28.3–57.6)	53	700.2	27	54.5 (41.1–69.1)	1.02 (0.61–1.71)	0.94	1.07 (0.62–1.86)	0.808
High (>57.6)	26	323.9	14	66.6 (44.5–87.0)	1.06 (0.56–2.00)	0.854	1.04 (0.51–2.14)	0.915
Serum zeaxanthin/lutein							*p* for trend	0.373
Low (<42.9)	54	640.8	32	63.6 (50.0–77.0)	1.00		1.00	
Medium (42.9–57.3)	52	669.4	26	54.1 (40.4–69.0)	0.79 (0.47–1.33)	0.372	0.88 (0.51–1.52)	0.645
High (>57.3)	36	501.9	14	57.6 (37.9–78.8)	0.55 (0.29–1.02)	0.059	0.76 (0.37–1.53)	0.435
Serum cryptoxanthin							*p* for trend	0.866
Low (<11.2)	62	727.3	36	67.4 (53.9–80.2)	1.00		1.00	
Medium (11.2–22.1)	47	644.3	20	48.4 (33.9–65.2)	0.63 (0.36–1.09)	0.098	0.72 (0.39–1.31)	0.279
High (>22.1)	33	440.5	16	53.9 (36.6–73.1)	0.73 (0.40–1.31)	0.286	0.85 (0.44–1.64)	0.63
Serum lycopene							*p* for trend	0.517
Low (<19.8)	43	543.8	21	55.3 (39.9–71.9)	1.00		1.00	
Medium (19.8–35.8)	55	761.7	29	60.8 (46.7–75.1)	0.96 (0.55–1.69)	0.896	0.79 (0.42–1.48)	0.457
High (>35.8)	44	506.6	22	54.4 (39.2–70.9)	1.08 (0.59–1.96)	0.802	0.77 (0.38–1.54)	0.456
Serum α-tocopherol							*p* for trend	0.042
Low (<753.0)	53	594.2	34	68.8 (55.5–81.4)	1.00		1.00	
Medium (753.0–983.9)	49	718.2	19	43.5 (30.1–59.7)	0.47 (0.27–0.83)	0.009	0.53 (0.27–0.94)	0.03
High (>983.9)	40	499.7	19	66.7 (46.0–86.0)	0.64 (0.36–1.11)	0.114	0.76 (0.42–1.40)	0.383
Retinol intake							*p* for trend	0.58
Low (<190.2)	50	573.8	29	62.3 (48.3–76.4)	1.00		1.00	
Medium (190.2–313.1)	51	673.9	25	56.5 (42.1–71.9)	0.74 (0.43–1.26)	0.263	0.76 (0.42–1.37)	0.36
High (>313.1)	41	564.4	18	52.3 (36.2–70.6)	0.63 (0.35–1.13)	0.124	0.57 (0.29–1.13)	0.106
Carotene intake							*p* for trend	0.182
Low (<3,281.4)	64	730.7	34	59.8 (46.9–73.1)	1.00		1.00	
Medium (3,281.4–5,042.8)	43	632.0	22	58.7 (42.7–75.4)	0.72 (0.42–1.24)	0.238	0.71 (0.39–1.31)	0.272
High (>5,042.8)	35	449.4	16	52.9 (35.8–72.2)	0.73 (0.41–1.33)	0.309	0.55 (0.25–1.18)	0.122
Vitamin A intake							*p* for trend	0.268
Low (<2,398.8)	65	723.6	36	61.9 (49.1–74.9)	1.00		1.00	
Medium (2,398.8–3,466.7)	43	642.5	19	49.1 (34.4–66.2)	0.59 (0.34–1.03)	0.064	0.58 (0.31–1.07)	0.081
High (>3,466.7)	34	446.0	17	60.6 (42.2–79.4)	0.74 (0.42–1.32)	0.307	0.60 (0.28–1.32)	0.208
Vitamin E intake							*p* for trend	0.567
Low (<6.7)	61	684.0	32	56.7 (44.1–70.1)	1.00		1.00	
Medium (6.7–8.7)	45	720.6	19	49.0 (34.4–66.0)	0.56 (0.32–0.99)	0.047	0.51 (0.25–1.05)	0.066
High (>8.7)	36	407.5	21	67.3 (49.6–83.8)	1.02 (0.59–1.77)	0.947	0.56 (0.23–1.38)	0.211

Cox's proportional hazard model showing the hazard ratio for regression in a cumulative 24-month period in current smokers only. The adjusted model was identical to the model used in Table 3. The units of micronutrients are expressed as μg/dL

Table 6 HR of progression from entire CIN1/2 according to the serum micronutrients and nutrient intake questionnaire

	n	Person-months	Events	Cumulative 5-year rate (95 % CI)	Hazard ratio for progression (95 % CI)			
					Unadjusted	*p* value	Adjusted model	*p* value
Serum retinol							*p* for trend	0.372
Low (<55.2)	128	4,588.2	7	8.7 (3.6–20.1)	1.00		1.00	
Medium (55.2–67.9)	132	5,048.8	17	17.1 (10.8–26.6)	2.25 (0.93–5.44)	0.071	2.35 (0.95–5.77)	0.063
High (>67.9)	131	5,210.1	14	14.3 (8.5–23.7)	1.82 (0.73–4.51)	0.198	2.23 (0.88–5.60)	0.089
Serum α-carotene							*p* for trend	0.669
Low (<5.1)	127	4,506.6	13	15.4 (8.7–26.2)	1.00		1.00	
Medium (5.1–9.7)	133	4,955.5	17	16.0 (10.0–25.0)	1.21 (0.59–2.49)	0.609	1.08 (0.51–2.31)	0.835
High (>9.7)	131	5,385.0	8	9.6 (4.7–19.0)	0.52 (0.22–1.27)	0.153	0.46 (0.18–1.15)	0.098
Serum β-carotene							*p* for trend	0.337
Low (<28.3)	129	4,245.0	18	21.8 (13.6–33.9)	1.00		1.00	
Medium (28.3–57.6)	131	5,208.1	7	7.0 (3.2–14.7)	0.32 (0.13–0.77)	0.011	0.28 (0.11–0.71)	0.007
High (>57.6)	131	5,394.0	13	13.2 (7.7–22.3)	0.58 (0.28–1.19)	0.14	0.52 (0.24–1.13)	0.098
Serum zeaxanthin/lutein							*p* for trend	0.772
Low (<42.9)	130	4,611.4	11	12.1 (6.7–21.4)	1.00		1.00	
Medium (42.9–57.3)	130	5,291.5	17	17.9 (11.2–28.0)	1.37 (0.64–2.94)	0.415	1.58 (0.71–3.53)	0.266
High (>57.3)	131	4,944.2	10	9.4 (5.1–17.1)	0.87 (0.37–2.06)	0.756	0.95 (0.39–2.32)	0.908
Serum cryptoxanthin							*p* for trend	0.618
Low (<11.2)	129	4,591.6	12	12.2 (6.9–20.9)	1.00		1.00	
Medium (11.2–22.1)	130	4,906.2	16	17.1 (10.6–27.0)	1.26 (0.60–2.67)	0.544	1.37 (0.61–3.06)	0.445
High (>22.1)	132	5,349.3	10	10.5 (5.5–19.7)	0.73 (0.32–1.69)	0.465	0.71 (0.29–1.72)	0.450
Serum lycopene							*p* for trend	0.286
Low (<19.8)	129	4,827.0	15	17.5 (10.5–28.3)	1.00		1.00	
Medium (19.8–35.8)	131	4,954.6	11	10.0 (5.6–17.6)	0.71 (0.33–1.55)	0.395	0.61 (0.27–1.36)	0.223
High (>35.8)	131	5,065.5	12	13.1 (7.3–22.9)	0.76 (0.36–1.63)	0.48	0.73 (0.33–1.59)	0.428
Serum α-tocopherol							*p* for trend	0.788
Low (<753.0)	128	5,143.1	11	12.0 (6.6–21.2)	1.00		1.00	
Medium (753.0–983.9)	132	5,052.6	11	13.3 (7.4–23.3)	1.01 (0.44–2.33)	0.983	0.91 (0.39–2.10)	0.820
High (>983.9)	131	4,651.4	16	15.7 (9.3–25.8)	1.60 (0.74–3.45)	0.232	1.87 (0.84–4.19)	0.126
Retinol intake							*p* for trend	0.666
Low (<190.2)	130	4,778.5	14	14.7 (8.6–24.4)	1.00		1.00	
Medium (190.2–313.1)	130	4,985.2	15	16.7 (9.8–27.7)	1.02 (0.49–2.12)	0.948	1.08 (0.51–2.32)	0.834
High (>313.1)	131	5,083.4	9	9.5 (4.9–17.7)	0.60 (0.26–1.40)	0.239	0.62 (0.23–1.68)	0.346
Carotene intake							*p* for trend	0.331
Low (<3,281.4)	130	4,578.9	9	10.8 (5.2–21.6)	1.00		1.00	
Medium (3,281.4–5,042.8)	131	4,789.0	16	17.6 (11.4–26.7)	2.02 (0.91–4.46)	0.083	2.30 (0.97–5.42)	0.058
High (>5,042.8)	130	5,479.2	10	11.6 (6.2–21.0)	0.94 (0.38–2.33)	0.901	1.19 (0.41–3.44)	0.746
Vitamin A intake							*p* for trend	0.493
Low (<2,398.8)	130	4,510.5	11	12.2 (6.3–22.9)	1.00		1.00	
Medium (2,398.8–3,466.7)	131	4,921.0	16	15.1 (9.4–23.9)	1.33 (0.62–2.87)	0.463	1.32 (0.59–2.97)	0.500
High (>3,466.7)	130	5,415.6	11	12.6 (3.8–22.2)	0.84 (0.36–1.95)	0.689	0.92 (0.33–2.54)	0.873
Vitamin E intake							*p* for trend	0.834
Low (<6.7)	130	4,431.0	12	13.8 (7.5–24.7)	1.00		1.00	
Medium (6.7–8.7)	130	5,128.1	15	14.1 (8.6–22.6)	1.08 (0.51–2.31)	0.842	1.06 (0.44–2.56)	0.892
High (>8.7)	131	5,288.0	11	12.5 (6.8–22.1)	0.78 (0.34–1.77)	0.55	1.00 (0.30–3.38)	0.998

Cox's proportional hazard model showing the hazard ratio for progression over a cumulative 60-month period. The adjusted model was identical to the model used in Table 3. The units of micronutrients are expressed as μg/dL

effects were weaker or not found with a higher level of serum beta-carotene (HR 0.52, 95 % CI 0.24–1.13, $p = 0.098$). In contrast, a high carotene intake did not show an inverse relationship, but rather a non-significant increase in progression (HR 2.30, 95 % CI 0.97–5.42, $p = 0.058$). There was no significant association between other serum micronutrients and risk for CIN progression.

Discussion

The role of environmental factors, including micronutrients and tobacco smoking, in cervical carcinogenesis has been discussed. Smoking status in particular interfered with serum levels and intake of carotenoids as shown in Tables 1 and 2. In smokers, food intake is intrinsically lower than in non-smokers [22]. From the questionnaires, the intake per day of all micronutrients, except retinol and tocopherol, was lower in current smokers than in non-smokers, suggesting an unbalanced diet resulting from either smoking or other lifestyle behaviors (Table 1). Serum levels of alpha-carotene, beta-carotene and cryptoxanthin were inversely correlated with smoking status, but alpha-tocopherol was not correlated with smoking status after adjusting for age, BMI and frequency of alcohol intake (Table 2). These data were consistent with a previous report in which smoking was shown to affect serum beta-carotene levels but to have no effect on alpha-tocopherol levels [23]. Though alpha-tocopherol and beta-carotene are well known as antioxidants, the antioxidant effect of alpha-tocopherol is not due to a reaction with oxygen. In contrast, beta-carotene does react with oxygen. This suggests that there is a difference in the mechanisms of antioxidant reaction [24].

In regression subjects, we expected to find a protective effect from high serum levels or intake of carotenoids; however, neither of these had protective effects. We assume that smoking status modulates dietary intake or serum levels of micronutrients. Therefore, we investigated the association between dietary intake or serum levels of micronutrients and CIN regression, taking into account smoking status (Tables 3, 4, 5). In non-smoking regression subjects, regression was significantly related to the serum levels of zeaxanthin/lutein. This relationship was not found in current smokers. In a similar example, an isoflavone has a protective effect for lung cancer, but the effect is abolished by smoking [25]. It was reported that zeaxanthin/lutein may be a useful marker of intake of leafy vegetables, spinach, green peas, broccoli and seaweed [26]. Zeaxanthin/lutein is chemically more hydrophilic than other carotenoids such as alpha- and beta-carotene, lycopene and beta-cryptoxanthin. The mechanisms of a potential protection against carcinogenesis may include: induction of apoptosis, inhibition of angiogenesis, enhancement of gap junction intercellular communication, induction of cell differentiation, prevention of oxidative damage, and modulation of the immune system. Serum levels of lutein have been inversely associated with cytochrome CYP1A2 activity, a hepatic enzyme responsible for the metabolic activity of a number of putative human carcinogens [27]. High serum levels of alpha-tocopherol tend to have an inhibitory effect on regression in smokers (Table 4). There is a similar effect in that supplemental vitamin E, presumably causing a high concentration of alpha-tocopherol, is associated with an increased risk of lung cancer, which was confined to current smokers [28]. Alpha-tocopherol is considered to be an antioxidant, but it might act as a pro-oxidant [24].

Though a weak and non-significant protective effect of dietary intake or low serum concentration of beta-carotene has been observed previously [10, 15, 29, 30], we found that a medium serum level of beta-carotene showed a significant protective effect on CIN progression, whereas this protective effect at higher serum levels of beta-carotene was weaker or abolished (Table 6). These data appear to be consistent with in-vitro experiments reporting that very high concentrations of beta-carotene decreased antioxidant and/or induced pro-oxidant effects [31, 32]. Based on epidemiological studies that have shown an association between a low intake of carotenes and human cancers [33], an intervention study was conducted for the prevention of lung cancer [34]. However, it was paradoxically reported that high serum levels of beta-carotene induced by oral supplements promoted lung cancer in male heavy smokers aged 50–69 years. In CIN, oral beta-carotene supplementation did not enhance CIN regression in a randomized, double-blind phase III trial [35]. One explanation for these failures may be that oral supplements induced extremely high serum levels of beta-carotene. Taken together, these data suggest that medium serum levels of beta-carotene may interfere with CIN progression or cancer development.

There was a discrepancy between the results of dietary intake and serum levels of beta-carotene. Endogenous metabolic processes may influence the serum concentrations of micronutrients. In fact, inconsistent results of the serum levels and dietary intake of alpha-tocopherol in patients with prostate cancer, and contradictory results of retinol in patients with cervical cancer, have been reported previously [14, 36, 37]. Additionally, there is limited dietary intake information obtained from questionnaires because of inherent recall bias. We examined the residual confounding factors, including passive smoking, the number of sexual partners, and serum *Chlamydia* IgG antibody, in addition to the adjusted model. Despite confounding by other risk factors included for adjustments, the analyses did not change the conclusion.

To our knowledge, this is the first large-scale prospective cohort study for CIN outcome to report an association between serum levels of antioxidant micronutrients adjusted for potential confounders including CIN grade, HPV genotype, age, total energy intake and smoking. To make our comparisons, we investigated not only serum levels but also dietary intake of micronutrients, despite the fact that food-intake questionnaires contain limited information. It is known that the accuracy of recalling past dietary intake is influenced by current dietary habits [38]. There are inconsistent results between previous case–control and cohort studies. However, our discrepant results did not reach the conclusion that women with CIN received a benefit from consuming a beta-carotene-rich diet. However, not smoking and maintaining high serum levels of zeaxanthin/lutein, presumably by intake of leafy vegetables, spinach, green peas, broccoli, and seaweed, are advantageous for the prevention of cervical cancer.

This study has some potential limitations. We included only CIN patients with an available serum sample for measurement of serum nutrients [18]. The majority of CIN patients already had persistent HPV infection at enrollment in the present study. If these nutrients play an important role in preventing persistent HPV infection, we cannot determine that role in this cohort study. The food intake contains not only the micronutrients being investigated but also other nutrients and mixtures. The incident number of progression cases was small and it was difficult to analyze by smoking status. A large-scale cohort study with a longer period of observation is required to clarify the association between serum levels or dietary intake of micronutrients and the risk of developing cervical cancer.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Syrjnen K, Hakama M, Saarikoski S et al (1990) Prevalence, incidence, and estimated life-time risk of cervical human papillomavirus infections in a nonselected Finnish female population. Sex Transm Dis 17:15–19
2. Schiffman M, Castle PE, Jeronimo J et al (2007) Human papillomavirus and cervical cancer. Lancet 370:890–907
3. Castellsague X, Bosch FX, Munoz N (2002) Environmental co-factors in HPV carcinogenesis. Virus Res 89:191–199
4. Jordan JA, Singer A (eds) (2006) The cervix, 2nd edn. Blackwell Publishing Ltd, Malden
5. Giuliano AR (2000) The role of nutrients in the prevention of cervical dysplasia and cancer. Nutrition 16:570–573
6. González CA, Travier N, Luján-Barroso L et al (2011) Dietary factors and in situ and invasive cervical cancer risk in the European prospective investigation into cancer and nutrition study. Int J Cancer 129:449–459
7. Ghosh C, Baker JA, Moysich KB et al (2008) Dietary intakes of selected nutrients and food groups and risk of cervical cancer. Nutr Cancer 60:331–341
8. Tomita LY, Longatto Filho A, Costa MC et al (2010) Diet and serum micronutrients in relation to cervical neoplasia and cancer among low-income Brazilian women. Int J Cancer 126:703–714
9. Cho H, Kim MK, Lee JK et al (2009) Relationship of serum antioxidant micronutrients and sociodemographic factors to cervical neoplasia: a case–control study. Clin Chem Lab Med 47:1005–1012
10. García-Closas R, Castellsagu X, Bosch X, González CA (2005) The role of diet and nutrition in cervical carcinogenesis: a review of recent evidence. Int J Cancer 117:629–637
11. Rock CL, Michael CW, Reynolds RK et al (2000) Prevention of cervix cancer. Crit Rev Oncol Hematol 33:169–185
12. Steinmetz KA, Potter JD (1996) Vegetables, fruit, and cancer prevention: a review. J Am Diet Assoc 96:1027–1039
13. Mascio P, Murphy M, Sies H (1991) Antioxidant defense systems: the role of carotenoids, tocopherols, and thiols. Am J Clin Nutr 53:194S–200S
14. Nagata C, Shimizu H, Higashiiwai H et al (1999) Serum retinol level and risk of subsequent cervical cancer in cases with cervical dysplasia. Cancer Invest 17:253–258
15. Sedjo RL, Papenfuss MR, Craft NE et al (2003) Effect of plasma micronutrients on clearance of oncogenic human papillomavirus (HPV) infection (United States). Cancer Causes Control 14:319–326
16. Nagata C, Shimizu H, Yoshikawa H et al (1999) Serum carotenoids and vitamins and risk of cervical dysplasia from a case–control study in Japan. Br J Cancer 81:1234–1237
17. Matsumoto K, Oki A, Furuta R et al (2010) Tobacco smoking and regression of low-grade cervical abnormalities. Cancer Sci 101:2065–2073
18. Matsumoto K, Oki A, Furuta R et al (2011) Predicting the progression of cervical precursor lesions by human papillomavirus genotyping: a prospective cohort study. Int J Cancer 128:2898–2910
19. Takatsuka N, Kurisu Y, Nagata C et al (1997) Validation of simplified diet history questionnaire. J Epidemiol 7:33–41
20. Yoshikawa H, Kawana T, Kitagawa K et al (1991) Detection and typing of multiple genital human papillomaviruses by DNA amplification with consensus primers. Jpn J Cancer Res 82:524–531
21. Miller KW, Yang CS (1985) An isocratic high-performance liquid chromatography method for the simultaneous analysis of plasma retinol, alpha-tocopherol, and various carotenoids. Anal Biochem 145:21–26
22. Tomita LY, Roteli-Martins CM, Villa LL et al (2011) Associations of dietary dark-green and deep-yellow vegetables and fruits with cervical intraepithelial neoplasia: modification by smoking. Br J Nutr 105:928–937
23. Palan PR, Mikhail MS, Basu J et al (1991) Plasma levels of antioxidant β-carotene and α-tocopherol in uterine cervix dysplasias and cancer. Nutr Cancer 15:13–20
24. Schneider C (2005) Chemistry and biology of vitamin E. Mol Nutr Food Res 49:7–30

25. Shimazu T, Inoue M, Sasazuki S et al (2010) Isoflavone intake and risk of lung cancer: a prospective cohort study in Japan. Am J Clin Nutr 91:722–728
26. Ito Y, Shimizu H, Yoshimura T et al (1999) Serum concentrations of carotenoids, alpha-tocopherol, fatty acids, and lipid peroxides among Japanese in Japan, and Japanese and Caucasians in the US. Int J Vitamin Nutr Res 69:385–395
27. Ribaya-Mercado JD, Blumberg JB (2004) Lutein and zeaxanthin and their potential roles in disease prevention. J Am Coll Nutr 23:567S–587S
28. Slatore CG, Littman AJ, Au DH et al (2008) Long-term use of supplemental multivitamins, vitamin C, vitamin E, and folate does not reduce the risk of lung cancer. Am J Respir Crit Care Med 177:524–530
29. Giuliano AR, Papenfuss M, Nour M et al (1997) Antioxidant nutrients: associations with persistent human papillomavirus infection. Cancer Epidemiol Biomarkers Prev 6:917–923
30. Giuliano AR, Siegel EM, Roe DJ et al (2003) Dietary intake and risk of persistent human papillomavirus (HPV) infection: the Ludwig–McGill HPV Natural History Study. J Infect Dis 188:1508–1516
31. El-Agamey A, Lowe GM, McGarvey DJ et al (2004) Carotenoid radical chemistry and antioxidant/pro-oxidant properties. Archiv Biochem Biophys 430:37–48
32. Burton GW, Ingold K (1984) Beta-carotene: an unusual type of lipid antioxidant. Science 224:569–573
33. Peto R, Doll R, Buckley JD et al (1981) Can dietary beta-carotene materially reduce human cancer rates? Nature 290:201–208
34. Alberts D, Barakat R (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. N Engl J Med 330:1029–1035
35. Keefe KA, Schell MJ, Brewer C et al (2001) A randomized, double blind, Phase III trial using oral beta-carotene supplementation for women with high-grade cervical intraepithelial neoplasia. Cancer Epidemiol Biomarkers Prev 10:1029–1035
36. Weinstein SJ, Wright ME, Lawson KA et al (2007) Serum and dietary vitamin E in relation to prostate cancer risk. Cancer Epidemiol Biomarkers Prev 16:1253–1259
37. Kanetsky PA, Gammon MD, Mandelblatt J et al (1998) Dietary intake and blood levels of lycopene: association with cervical dysplasia among non-Hispanic, black women. Nutr Cancer 31:31–40
38. Myung SK, Ju W, Kim SC et al (2011) Vitamin or antioxidant intake (or serum level) and risk of cervical neoplasm: a meta-analysis. BJOG 118:1285–1291

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Effective treatment of pelvic lymphocele by lymphaticovenular anastomosis

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HIGHLIGHTS

- Pelvic lymphocele is a major complication after pelvic lymphadenectomy.
- We performed lymphaticovenular anastomosis (LVA) on pelvic lymphoceles, and found that LVA was highly effective regardless of the lymphoceles' size.
- LVA could be considered as an initial treatment for lymphoceles.

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ABSTRACT

Objective. Pelvic lymphocele can be a severe complication associated with surgical procedures such as pelvic lymphadenectomy. Lymphaticovenular anastomosis (LVA) is increasing in popularity as a surgical treatment for lymphedema. The aim of this study was to evaluate whether LVA is an effective treatment for lymphocele, which is caused by an obstruction of the lymphatic flow in a manner similar to the development of lymphedema.

Methods. Eleven female patients, who presented with lymphocele, were treated with LVA. Before the operation, 3 of them were treated with a percutaneous catheter. Lymphocele size and the volume of daily drainage were measured before and after LVA.

Results. The lymphocele was completely resolved in 6 patients and partially resolved in the remaining 5 patients. The mean size of the pelvic lymphocele changed from 400 ml (range 50–1050 ml) to 43 ml (range 0–120 ml) ($P<0.01$). In the 3 patients who had percutaneous drainage catheters, the volume of fluid drained decreased from 340 ml/day to 20 ml/day after LVA.

Conclusions. Our technique is minimally invasive and is performed under local anesthesia. LVA is effective regardless of the size of the lymphocele. Therefore, LVA should be considered as a therapy for lymphocele because of its low invasiveness and its effectiveness in re-establishing circulation of lymphatic flow. Further studies should be performed to compare LVA with other minimally invasive techniques, such as percutaneous catheter and sclerotherapy.

Introduction

A lymphocele is defined as an abnormal collection of lymph fluid, without an epithelial lining, at the site of lymphatic surgery [1,2]. A pelvic lymphocele can occur after surgical procedures such as pelvic lymphadenectomy for gynecologic or prostatic malignancies and renal transplantation [2–7] and has an incidence of 1–49% [1,2,8]. Most lymphoceles are small and asymptomatic, and they disappear spontaneously with time. However, when sufficiently large, they may lead to symptoms such as abdominal pain, infection, increased urinary frequency, hydronephrosis, deep venous thrombosis, and lower extremity lymphedema [1,2,6,8].

Several treatment options are available for the management of pelvic lymphoceles; however, there is no consensus as to which is most effective. Needle aspiration and percutaneous catheter drainage, which are commonly used in the initial management of symptomatic lymphoceles, have reported initial cure rates of up to 80%, but treated lymphoceles are often complicated by infection (in up to 50% of cases) and recur in 80–90% of cases [1,2,9]. The cure rate of sclerotherapy is also reported to be between 77% and 98%, but the success of this treatment is inversely proportional to the size of the lymphocele—larger lymphoceles are more likely to be symptomatic and cause complications; thus, the effectiveness of this therapy is limited [1,9]. Laparoscopic or surgical fenestration is the most invasive of the current therapies

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for pelvic lymphoceles and is often reserved for refractory cases. Complications associated with this approach include perforation of the bladder, transection of the ureter, and injury of pelvic vessels [6,10].

Lymphaticovenular anastomosis (LVA) using supermicrosurgery has been reported as being a simple, minimally invasive, and effective treatment for secondary lymphedema of the upper and lower extremities [11–15]. This technique bypasses proximal lymphatic blockages, providing an alternative route for lymphatic fluid recirculation into the venous system. We reasoned that a similar principle could be used to treat pelvic lymphoceles. By providing an alternative route of lymphatic drainage into the venous system for lymphatic fluid from the lower limb, the flow of lymphatic fluid into the lymphocele would be reduced. Furthermore, we have previously demonstrated that valvular incompetence permits a reversal of the lymphatic flow in cases of lymphedema, and we hypothesized that a similar mechanism in the postsurgical pelvic lymphatic system of patients with lymphoceles would allow drainage of the lymphocele through the newly created LVAs. We have previously reported successful management of a pelvic lymphocele using this approach in a single patient [16]. In this study, we report our experience using this technique to treat pelvic lymphoceles in a series of 11 patients.

Materials and methods

Patients

Eleven female patients with pelvic lymphoceles were referred to our department between May 2010 and October 2011. All the patients had undergone treatment for gynecologic cancer (see Table 1). The presence of a pelvic lymphocele was determined in all cases by a CT scan. In 3 patients, a percutaneous catheter had been inserted prior to referral in an attempt to treat the lymphocele, but drainage had remained unacceptably high.

Preoperative preparation

All patients gave fully informed consent for the procedure, acknowledging that current outcome data on efficacy was unknown. One day before each operation, fluorescence lymphatic imaging, using a near-infrared fluorescence imaging device (Photodynamic Eye, Hamamatsu Photonics, Hamamatsu City, Japan), was performed after the injection of indocyanine green dye (ICG) to identify the lymphatic channels in both lower limbs, as previously described [17–19]. The location of the lymphatic channels was marked, facilitating the accurate placement of short incisions and thereby allowing the procedure to be performed under local anesthesia. In those patients with a percutaneous drainage device in situ, the drain was clamped after lymphatic mapping in order to increase pressure in the lower-limb lymphatics and facilitate LVA.

Operative technique

Under local anesthesia, 2 or 3 incisions (2 cm each) were made on each lower limb—on the dorsum of the foot, the distal medial thigh, and the groin—overlying previously mapped lymphatic channels [20]. Dissection of superficial lymphatic channels and venules was performed under magnification using the operative microscope. Lymphaticovenular anastomosis was performed using either 11/0 or 12/0 nylon sutures in an end-to-end (Fig. 1A and B) or side-to-end configuration. The patency of anastomoses was confirmed by either washout of the venous lumen by lymphatic flow or venous backflow into the lymphatic channels. Wounds were closed with intradermal 4/0 PDS and interrupted 5/0 nylon sutures.

Postoperative management

Twice daily for 7 days after surgery, 60 μg of prostaglandin E1 (Prostandin; Ono Pharma. Co., Osaka, Japan) was injected intravenously. Prostaglandin is used for dilation of the vessels and seems to result in decreased occlusion of the anastomosis site. Compression therapy was started on postoperative day 14. All but one patient had follow-up CT scans.

Assessment

Assessment of the lymphocele was performed by either CT or ultrasonography. The volume of the lymphocele was calculated as an ellipsoid. Statistical analysis of the data was performed using a Wilcoxon test. A P value less than 0.05 was deemed significant.

Results

The demographic details of the patients and the details of their gynecologic treatment are shown in Table 1. We performed a mean of 8.2 lymphaticovenular anastomoses, with a mean venule diameter of 0.70 mm and a mean lymphatic diameter of 0.55 mm. In 6 of the 11 patients, the pelvic lymphocele was completely resolved after LVA, and in the remaining 5 patients, the lymphocele was partially resolved. The average pelvic lymphocele size was 400 ml (range 50–1050 ml) on preoperative CT scan and 43 ml (range 0–120 ml) on postoperative CT ($P<0.01$). In the 3 patients who underwent preoperative placement of percutaneous drainage catheters, the mean volume of fluid drained each day was reduced from 340 ml to 20 ml after LVA (Fig. 2).

Prior to our operations, 10 patients had symptoms: 1 had hydronephrosis requiring a urinary stent, 1 had frequent pre-ileus, 2 had increased urinary frequency, 2 had infection of lymphoceles, 3 had abdominal pain, and 7 had lower-extremity lymphedema. All symptoms except for lymphedema were alleviated after the LVA operation, and the lymphedema was improved from the pre-LVA state. No patients in

Table 1
Patient data.

Age	Site of primary cancer	Stage	Primary operation	Pre-LVA lymphocele volume [ml]	Post-LVA lymphocele volume [ml]	Catheter inserted
52	Cervical cancer	Ib	RH, BSO, PLA	60	0	−
63	Cervical cancer	IVb	RH, SILA, PALA	160	0	+
42	Endometrial cancer	Ic	RH, BSO, PLA	50	0	−
53	Endometrial cancer	Ib	RH, BSO, PLA	1050	0	+
53	Endometrial cancer	Ic	TAH, BSO, PLA	700	0	−
66	Endometrial cancer	Ic	RH, BSO, PLA, PALA	200	110	−
42	Ovarian cancer	Ic	TAH, BSO, PLA, PALA,	460	50	−
53	Ovarian cancer	Ia	TAH, BSO, PLA, PALA, pOM	170	90	−
56	Ovarian cancer	IIIc	Secondary EILA	500	100	+
61	Ovarian cancer	Ic	TAH, BSO, PLA, PALA, pOM	700	0	−
69	Ovarian cancer	Ic	TAH, BSO, PLA, PALA, pOM	350	120	−

RH: radical hysterectomy, BSO: bilateral salpingo-oophorectomy, PLA: pelvic lymphadenectomy, SILA: superficial inguinal lymphadenectomy, TAH: abdominal total hysterectomy, pOM: partial omentectomy, EILA: external iliac lymphadenectomy, PALA: para-aortic lymphadenectomy.

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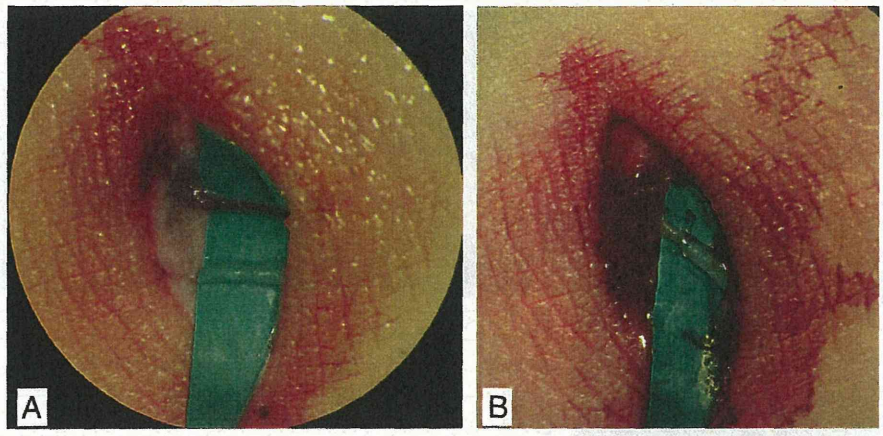

Fig. 1. Lymphaticovenular anastomosis. (A) Pre-anastomosis image. The upper vessel is a vein; the lower image is a lymphatic channel. (B) Post-anastomosis image. The anastomosis was performed with 5 sutures of 11–0 nylon. The left side of the anastomosis looks clear because the lymphatic fluid is under higher pressure than venous blood; therefore, flow from the lymphatic channel on the right washes out blood from inside the vein.

our series suffered any complications of LVA. Specifically, there were no infections and no wound-healing problems.

Representative case

The lymphocele was detected on CT 11 days before LVA (Fig. 3A). The patient had abdominal pain, urinary frequency, and lower-extremity lymphedema. A percutaneous catheter was inserted 3 days before the operation and the daily volume of drained fluid was recorded until the tube was removed (Fig. 3D). The operation site of LVA was noted (Fig. 3B). A CT image taken 3 days after the operation showed that the lymphocele had disappeared (Fig. 3C). The catheter was removed after confirming that the symptoms had disappeared.

Discussion

Since Teruel et al. [21] first reported successful sclerotherapy with povidone iodine for lymphoceles, several types of sclerotherapy with a variety of agents have been reported [1,6,9]. The cure rate for sclerotherapy is reported to be between 77% and 98% [1], and the recurrence rate is 31% [22]. However, the success of this treatment is inversely proportional to the size of the lymphocele [1]—larger lymphoceles are more likely to be symptomatic and cause complications; thus, when the lymphoceles most require treatment, this therapy is likely to be relatively less effective.

Laparoscopic or open surgical fenestration can be used to open a pathway from the lymphocele into the peritoneal cavity, allowing the peritoneum to absorb lymphatic fluid [23]. These techniques enable lymphatic fluid to re-circulate into the venous system. However, they are more invasive than other therapies and have been associated with complications including bladder perforation, ureter transection, and injury of pelvic vessels [6,10]. Recurrence can occur with closure of the fenestrated window in 6–15% of cases [6,24].

The ideal therapy for lymphoceles would be more effective and less invasive than traditional treatment methods (including sclerotherapy and surgical fenestration), with fewer complications and a lower chance of recurrence. Moreover, restoration of lymphatic circulation, broken by lymphadenectomy, is desired.

LVA is emerging as the treatment of choice for lymphedema of the extremities. Before the LVA operation was available, only conservative therapies, such as massage and compression garments, could be used for lymphedema. These techniques do not enable re-establishment of lymphatic fluid circulation into the venous system, but simply release it into the trunk lesion. Therefore, patients are never able to discontinue the therapy if they wish to reduce the edematous lesion. LVA was

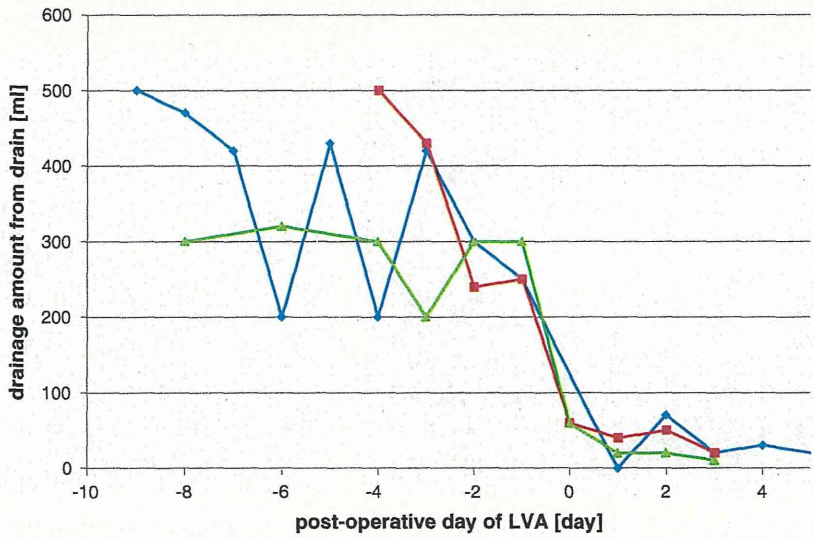

Fig. 2. Daily drainage from 3 patients who had preoperative placement of percutaneous drains. Note the dramatic decrease in drainage following LVA.

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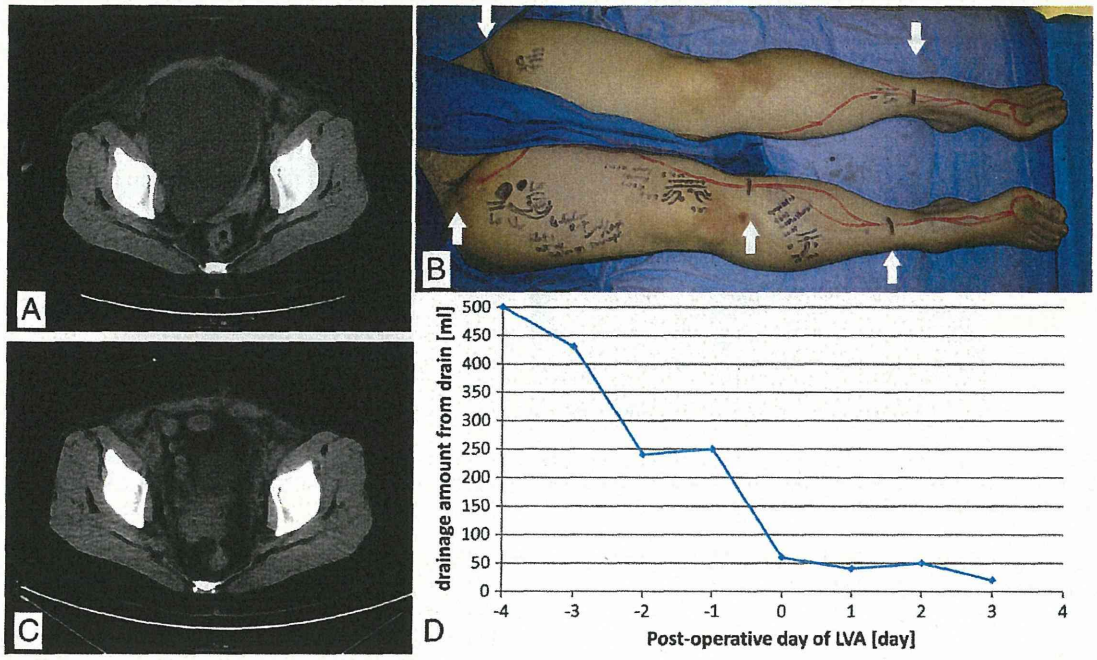

Fig. 3. Resolution of pelvic lymphocele after LVA. (A) CT scan of a large pelvic lymphocele after gynecologic surgery. (B) Immediate postoperative view. Seven anastomoses were performed through five 3-cm incisions under local anesthesia. (C) A CT scan of the same patient 3 days after LVA demonstrates complete resolution of the lymphocele. (D) The drainage chart of the same patient demonstrates large daily drainage volumes before LVA. Following LVA, the volume of fluid drained was dramatically reduced, and the drain was removed on postoperative day 4.

introduced as a new concept for lymphedema therapy [13–15]. The aim is to bypass proximal lymphatic blockages that cause congestion of lymphatic flow and thereby provide an alternative route for lymphatic fluid recirculation. Although the lymphatic channels normally have autokinetic movement because of smooth muscles, when the muscle damage due to lymphedema is irreversible, compression therapy is needed as an adjuvant therapy to direct lymphatic fluid into venulae. However, when the damage is dormant, the muscles react by pushing lymphatic fluid into the venous system. In this case, the patients do not need to receive any further adjuvant therapy.

In lymphoceles, the lymphatic flow from the lower limbs is similarly interrupted at the surgical region, where it flows into the cavity. This is illustrated in Fig. 4, where ICG injected into the dorsum of the feet is seen to escape into the percutaneous drainage catheter of a lymphocele. We reasoned that LVA would enable the lymphatic flow from the limbs to bypass the lymphocele, reducing its volume and preventing lymphatic flow into the lymphocele, thereby allowing spontaneous resolution. Our results supported this hypothesis, with total recovery in 6 of the 11 cases and improvement in the remaining 5 cases.

We believe that LVA has multiple advantages over the other methods currently used to treat lymphoceles. First, LVA is minimally invasive because it can be performed under local anesthesia and requires only 2 or 3 small skin incisions. Second, LVA is effective for

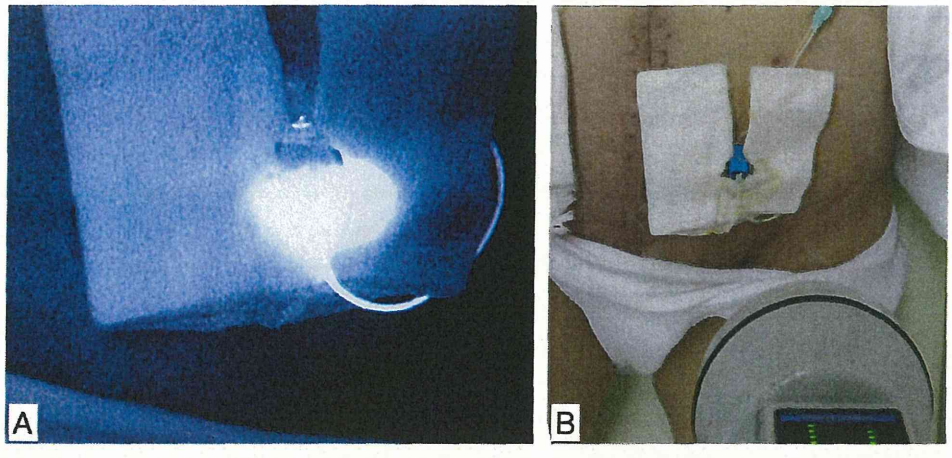

Fig. 4. Demonstration of lymphatic flow from the leg into a pelvic lymphocele. (A) Fluorescence lymphatic image of a percutaneous catheter, 5 min after injecting ICG into the first dorsal web space of the foot, indicates the lymphatic flow from the leg rapidly entering the lymphocele. It also indicates that the fluorescing root is the dominant lymphatic channel pouring into the lymphocele. (B) Conventional photograph of the same area. The Photodynamic Eye camera used to obtain the picture in (A) is seen at the bottom of the picture.

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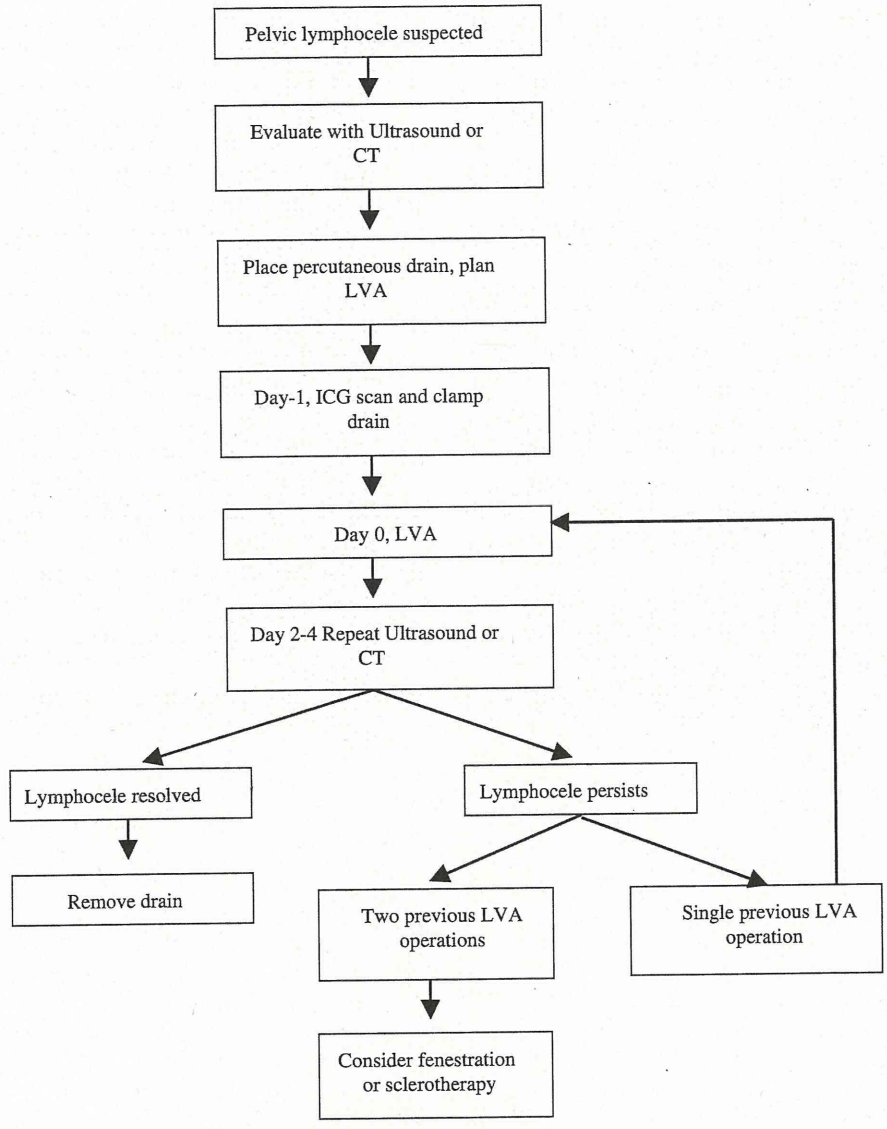

Fig. 5. Algorithm for the management of pelvic lymphocele.

all sizes of lymphocele. Third, LVA can prevent or improve lymphedema, which is a common complication of pelvic lymphadenectomy. This is in contrast to other techniques that resolve the fluid collection by blocking or sclerosing lymphatic channels, which may in itself provoke the development of lymphedema.

Our experience of reverse lymphatic flow with the valvular incompetence of lymphatic channels in lymphedema indicates that lymphatic flow into the lymphocele from places other than the leg may occur in a retrograde pattern into the leg's lymphatic channels and then into the venous system. Competent lymphatic valves may account for the partial failure of our technique, and we recommend that a percutaneous catheter be used to drain the remaining fluid if it is symptomatic.

Unfortunately, LVA is not perfectly effective for all patients. We suggest that the reason for this is that the lymphatic channels used for LVA are sometimes not the dominant lymphatic channels for the lymphoceles. In such cases, the lymphocele could diminish but not vanish. A second LVA might be able to locate the dominant lymphatic channel. Other therapies could also be used: LVA is an indirect approach to the lymphocele whereas other therapies approach lymphoceles directly. We present our algorithm for management of pelvic lymphoceles in Fig. 5.

In conclusion, our technique is minimally invasive and is performed under local anesthesia. It is therefore suitable for patients who have recently undergone major pelvic surgery. LVA should be considered as an initial therapy for lymphoceles because of its low invasiveness, high effectiveness, and ability to re-establish circulation of lymphatic flow. Further studies should be performed to compare LVA with other minimally invasive techniques, such as percutaneous catheter and sclerotherapy.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

[1] Mahrer A, Ramchandani P, Trerotola SO, Shlansky-Goldberg RD, Itkin M. Sclerotherapy in the management of postoperative lymphocele. J Vasc Interv Radiol 2010;21:1050-3.

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