Appendix 1

Supplementary methods

Method 1: identification and visualization of RLN by ultrasound

Due to dehydration and tissue degeneration of the cadaver, we could not confirm the RLN on the necropsy autopsy. Additionally, as it impossible to label RLN directly on patients, we used the following two solutions:

(I) Carbon nanoparticle injection: we selected 1 lymph node adjacent to the RLN in patients for injection of 0.1 mL carbon nanoparticles (Lai Mei Pharmaceutical Co., Chongqing, China) and the interval time from injection to surgery was less than 24 hours. The marked lymph nodes, recognized during surgery, proved to be adjacent to the RLN identified by preoperative ultrasound, as expected.

(II) Repeatability of RLN visualization: pre- and intra-operative measurements, intra- and inter-observer measurements were assessed using single-measure intraclass correlation coefficients (ICC). The concordance of results between preoperative and intraoperative ultrasound parameters was moderate (ICC 0.403). The ICCs for measurements taken independently by 2 ultrasonographers ranged from 0.495 to 0.824.

Method 2: preoperative ultrasound examination of the RLN

The ultrasound probe was placed at the paratracheal area, perpendicular to the coronal body plane. The operator started to scan the RLNs which were identified with the transducer in a transverse position, and color Doppler mode was used to distinguish the RLNs from small blood vessels. From this position, the nerve was tracked upwards to the entry of the laryngeal area and downwards to the supraclavicular area, with slight adjustments of the scanning plane. Finally, the ultrasound transducer was rotated into a longitudinal position with respect to the long axis in order to further identify thyroid lesions and the RLNs. The same steps were performed on the other side.

Method 3: appearance of normal nerves in ultrasound images

Normal peripheral nerves show a "honeycomb-like" appearance along the short axis, related to the presence of hypoechoic axons arranged in fascicles and multiple layers of hyperechoic connective tissue surrounding the axon bundles. The nerve appears as an elongated structure along the long axis, with alternating hypo- and hyper-echoic bands.

Method 4: exposure of the RLN during surgery

The borders of the exposed RLN were as follows: upper, the laryngeal entry point; lower, the innominate artery; medial, the trachea; and lateral, the inner edge of the common carotid artery.

Table S1 Univariate analysis to identify factors associated with RLN invasion

Factor	Category	No RLN invasion (n=736)	RLN invasion (n=80)	P value
Sex	Male	176 (75.00)	28 (25.00)	0.041
	Female	560 (85.64)	52 (14.36)	
Age, years	<30	132 (90.12)	8 (9.88)	<0.001
	30 to <40	279 (88.24)	20 (11.76)	
	40 to <50	155 (83.51)	16 (16.49)	
	50 to <60	118 (82.67)	13 (17.33)	
	≥60	52 (54.9)	23 (45.1)	
Body mass index, kg/m^2	<18.5	59 (82.05)	7 (17.95)	0.008
	18.5 to <24	449 (87.18)	35 (12.82)	
	≥24	228 (76.54)	38 (23.46)	
Tumor location	Diffuse	30 (54.54)	22 (45.46)	<0.001
	Isthmic	22 (100.00)	0 (0.00)	
	Upper	118 (95.16)	6 (4.84)	
	Middle	338 (89.65)	39 (10.35)	
	Lower	228 (94.61)	13 (5.39)	
Tumor size, mm	≤10	442 (93.68)	16 (6.32)	<0.001
	>10–20	184 (79.03)	26 (20.97)	
	>20–40	88 (70.59)	20 (29.41)	
	>40	22 (37.93)	18 (62.07)	
Tumor margin	Smooth	37 (91.30)	2 (8.70)	0.417
	Ill-defined	669 (82.71)	78 (17.29)	
Tumor shape	Regular	37 (95.45)	1 (4.55)	0.125
	Irregular	699 (82.52)	79 (17.48)	
Calcification	Macro	22 (71.43)	4 (38.57)	0.025
	Micro	441 (86.81)	36 (13.19)	
	Mixed	206 (76.35)	35 (23.65)	
	None	67 (87.18)	5 (12.82)	
Tumor adjacent to anterior thyroid capsule	Yes	236 (77.58)	37 (22.42)	0.013
	No	500 (98.13)	4 (1.87)	
Tumor adjacent to posterior thyroid capsule	Yes	346 (70.77)	76 (29.23)	<0.001
	No	390 (98.13)	4 (1.87)	
Tumor adjacent to medial thyroid capsule	Yes	213 (68.45)	53 (31.55)	<0.001
	No	523 (91.18)	27 (8.82)	
Tumor adjacent to lateral thyroid capsule	Yes	177 (70.9)	39 (29.1)	<0.001
	No	559 (87.94)	41 (12.06)	
Distance <1 mm between tumor and RLN	Yes	206 (58.64)	79 (41.36)	<0.001
	No	530 (99.65)	1 (0.35)	
Loss of typical fascicular echotexture of the RLN	Yes	29 (17.89)	78 (82.11)	<0.001
	No	707 (99.47)	2 (0.53)	
Loss of the echotexture of RLN epineurium	No	707 (99.47)	2 (0.53)	<0.001
	Yes	29 (17.89)	78 (82.11)	
Loss of the echotexture of RLN fibers and perineurium	Yes	8 (1.47)	67 (98.53)	<0.001
	No	728 (96.80)	13 (3.20)	

Values are n (%), unless otherwise indicated. RLN, recurrent laryngeal nerve.