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安宅, 映里

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**Cumulative smoking dose is associated with subclinical renal injury: a pathological study
in individuals without chronic kidney disease**

Eri Ataka¹, Yuta Matsukuma¹, Kenji Ueki¹, Akihiro Tsuchimoto¹, Yasuhiro Okabe², Kosuke
Masutani³, Masafumi Nakamura², Toshiaki Nakano^{1,4}, Takanari Kitazono¹

¹Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu
University, Fukuoka, Japan

²Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu
University, Fukuoka, Japan

³Department of Nephrology and Rheumatology, Department of Internal Medicine, Faculty of
Medicine, Fukuoka University, Fukuoka, Japan

⁴Center for Cohort Studies, Graduate School of Medical Sciences, Kyushu University,
Fukuoka, Japan

Running head: Smoking and subclinical renal injury

Corresponding author

Toshiaki Nakano, MD, PhD

Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu
University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan.

Phone: +81-92-642-5256

Fax: +81-92-642-5271

E-mail: nakano.toshiaki.455@m.kyushu-u.ac.jp

Abstract

Background: Epidemiological studies have identified smoking as an independent risk factor for development of chronic kidney disease. However, the early renal pathological lesions have not been clearly elucidated.

Methods: We investigated time-zero biopsy specimens from 547 living kidney donors and evaluated the relationships between smoking and renal histological changes, including arteriolar hyalinization, intimal thickening of small-medium arteries, global glomerulosclerosis, and interstitial fibrosis and tubular atrophy (IF/TA).

Results: A total of 199 subjects (36.4%) had smoking history, of whom 92 (16.8%) and 107 (19.6%) had <20 pack-years and ≥ 20 pack-years of smoking, respectively. Cumulative smoking dose was significantly associated with prevalence of arteriolar hyalinization: the multivariable-adjusted odds ratio (OR) per 20 pack-year increase was 1.50 [95% confidence interval 1.15–1.97]. The ORs for smokers with <20 pack-years and ≥ 20 pack-years versus never-smokers were 1.76 [1.01–3.09] and 2.56 [1.48–4.44], respectively. Smoking was also associated with prevalence of >10% global glomerulosclerosis: the OR per 20 pack-year increase was 1.24 [0.96–1.59]. The ORs for smokers with <20 pack-years and ≥ 20 pack-years versus never-smokers were 1.50 [0.98–2.78] and 2.11 [1.18–3.79], respectively. The ORs for these pathological changes increased significantly depending on cumulative smoking dose. Intimal thickening of small-medium arteries and IF/TA were not associated with smoking status. The prevalence of arteriolar hyalinization remained higher in patients with ≥ 10 years since smoking cessation than in never-smokers (OR 2.23 [1.03–4.83]).

Conclusions: Subclinical pathological injury caused by smoking is potentially associated with renal arteriolar hyalinization and glomerular ischaemia.

KEY LEARNING POINTS

What is already known about this subject?

- Smoking is an independent risk factor for development and progression of chronic kidney disease (CKD).
- Previous biopsy studies demonstrated an association between smoking and arteriosclerotic change, but the pathological features related to smoking in individuals without CKD remained unclear.

What this study adds?

- We investigated time-zero biopsy specimens from 547 living kidney donors without CKD, including 199 (36.4%) with smoking history. Cumulative smoking dose was significantly associated with prevalences of arteriolar hyalinization and abnormal glomerulosclerosis, even after adjustment for multiple atherosclerotic risk factors.
- There were dose-dependent relationships between smoking and these pathological changes, and the OR for arteriolar hyalinization remained higher in patients with ≥ 10 years since smoking cessation than in never-smokers.

What impact this may have on practice or policy?

- Smoking is an independent risk factor for subclinical arteriosclerosis in kidney pathology.
- This study emphasizes that smoking is potentially associated with kidney injury via vascular damage even in relatively healthy individuals.

Keywords: Cigarette smoking, hyaline, arteriosclerosis, kidney transplantation

Introduction

Several studies have identified cigarette smoking as a risk factor for development of chronic kidney disease (CKD) in the general population (1-5). Furthermore, a dose-dependent association between smoking and progression to advanced kidney failure was found in the general population (6, 7). Similar associations were observed in patients with various types of chronic glomerulonephritis (8, 9). A dose-response relationship between smoking pack-years and albuminuria was also identified (10). Meanwhile, two studies indicated that smoking cessation can reduce the risk of renal failure development in men (6, 7).

Despite the clear effects of smoking on CKD in clinical settings, the pathogenic mechanisms for the effects of smoking on progression of kidney disease, especially in the general population, have not been clearly elucidated. Several studies that examined autopsy (11, 12) and renal biopsy (13) specimens revealed associations between smoking and renal pathological changes, such as arteriosclerotic changes and global glomerulosclerosis. However, samples from renal biopsies may be affected by co-existing glomerular disease, and the autopsy studies involved relatively small numbers of study subjects. In addition, information on dose-dependent relationships was lacking. To clarify the associations between smoking and renal pathological changes in the general population more precisely, studies on samples from relatively healthy subjects are desirable.

In the present study, we examined the associations between cigarette smoking and renal pathological changes in individuals without primary kidney disease or proteinuria, by examining specimens from kidney donors who underwent a renal biopsy at the time of donation surgery. We also investigated whether there were dose-dependent relationships between smoking amount and renal pathological changes, and whether number of smoking cessation years was associated with renal arteriosclerotic lesions.

Materials and Methods

Study design and population

This cross-sectional study extracted data for 684 living kidney donors who underwent donation surgery and had a time-zero biopsy taken at Kyushu University, Fukuoka, Japan, from January 2006 to December 2018. Patients were excluded if they met one or more of the following criteria: [1] inadequate biopsy sample containing <10 glomeruli or small cortex of <2 mm²; [2] presence of compression artifacts; [3] missing data for relevant laboratory variables. After application of these criteria, 75 patients were excluded for reasons [1] and/or [2], and 62 were excluded for reason [3]. The remaining 547 donors were included in the study.

The study protocol was approved by the Human Ethics Committee of Kyushu University Hospital (protocol 21180-02), and the study was performed in accordance with the guidelines of the Declaration of Helsinki. The Ethics Committee waived the requirement for written informed consent due to the retrospective nature of the study.

Measurements

Baseline clinical data were obtained from medical records. Smoking and alcohol drinking habits were based on self-reported standard questionnaires, which all patients had to answer at the time of admission for surgery. Smoking cessation was required for all current smokers at the preoperative appointment. Total smoking history was estimated in pack-years, defined as the number of years of smoking 20 cigarettes (full pack) per day. Number of years after smoking cessation was also investigated. Smoking status was classified into three groups: never-smokers, smokers with <20 pack-years, and smokers with ≥20 pack-years. Number of smoking years and number of cigarettes per day were analyzed separately. Alcohol drinking habit was defined as current habitual drinking. Evaluation of complications was described in detail in our previous study (14). All donors were aged ≥20 years. Hypertension was defined as blood pressure ≥140/90 mmHg or use of anti-hypertensive agents. Diabetes mellitus was

defined as fasting plasma glucose ≥ 126 mg/dL, haemoglobin A1c $\geq 6.5\%$, or use of oral hypoglycaemic agents. If haemoglobin A1c was measured by the Japanese Diabetes Society value, 0.4% was added to equal the National Glycohemoglobin Standardization Program value (15). Hypercholesterolaemia was defined as serum total cholesterol ≥ 220 mg/dL (16). Hyperuricaemia was defined as serum uric acid > 7.0 mg/dL in men or > 6.0 mg/dL in women. Obesity was defined as body mass index (BMI) ≥ 25 kg/m². Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation multiplied by a Japanese coefficient of 0.813 (17) as follows: $eGFR = 0.813 \times 141 \times \min(\text{serum creatinine}/\kappa, 1)^\alpha \times \max(\text{serum creatinine}/\kappa, 1)^{-1.209} \times 0.993^{\text{age}} \times 1.018$ [if female], where κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of serum creatinine/ κ or 1, and max indicates the maximum of serum creatinine/ κ or 1.

Histopathological evaluation of time-zero biopsy specimens

A wedge sample from the outer cortex was taken at the time of donation. The tissue specimen was fixed in formalin and embedded in paraffin. Sections were cut at 2- μ m thickness and stained with haematoxylin-eosin, periodic acid-Schiff, Masson trichrome, and periodic acid-methenamine silver. The following pathological changes were evaluated: (1) arteriosclerotic changes (arteriolar hyalinization, intimal thickening of small–medium arteries); (2) global glomerulosclerosis; (3) interstitial fibrosis and tubular atrophy (IF/TA). Arteriolar hyalinization was graded according to the Banff classification (18): ah0, no PAS-positive hyaline arteriolar thickening; ah1, mild to moderate PAS-positive hyaline thickening in at least one arteriole; ah2, moderate to severe PAS-positive hyaline thickening in more than one arteriole; ah3, severe PAS-positive hyaline thickening in many arterioles. Representative images of the four grades are shown in Figure 1. The prevalence and proportion of hyalinization were evaluated according to severity (ah0, ah1, $\geq$ ah2). Small–medium arteries were determined

by presence of at least 100- μ m diameter plus internal elastic plate, and the percentage of luminal stenosis caused by intimal thickening of these arteries was calculated, as shown in Figure 2. The most severe lesion in the cut plane of the short axis of the artery in each sample was evaluated (19). We investigated the presence or absence of intimal thickening and the prevalence of more severe lesions (no lesion, 1%–49% luminal stenosis, $\geq$ 50% luminal stenosis). Global glomerulosclerosis was characterized by the percentage of globally sclerotic glomeruli in the total glomeruli. Histological abnormality of global glomerulosclerosis was defined $>10\%$ (20). IF/TA was evaluated as the percentage of IF/TA area relative to the whole cortex area in the biopsy section. The IF/TA area and whole cortex area were measured as shown in Figure 3 (21). To calculate the percentage of IF/TA, the IF/TA area was divided by the whole cortex area, and $>5\%$ IF/TA was defined as an abnormal change (22). Measurements for the percentages of luminal stenosis of small–medium arteries and IF/TA in the whole cortex were performed using the digital ruler in an image analysis software program (Evident cellSens Standard V3.1; Evident, Tokyo, Japan; <https://www.evidentscientific.com/ja/>). Two observers (EA and YM) evaluated the biopsy specimens while blinded to the donor characteristics.

Statistical analysis

All statistical analyses were performed with JMP version 16.0.0 (SAS Institute Inc., Cary, NC, USA) and R software version 4.2.1 (<https://www.R-project.org>). Data were expressed as frequency and percentage or median and interquartile range (IQR), as appropriate. The Jonckheere–Terpstra and Cochran–Armitage tests were used to examine the trends in the means and frequencies of covariates and pathological changes according to smoking status. The Dunnett t-test was used to evaluate the pathological changes by reference to never-smokers. Age and sex-adjusted and multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression analyses for the pathological lesions according to smoking status. Analyses were also performed by number of

171 smoking years and number of cigarettes smoked per day. Subgroup analyses were conducted
172 according to baseline characteristics. A two-tailed value of $p < 0.05$ was considered to indicate
173 statistical significance.

Results

Baseline characteristics of the patients according to smoking status

Among the 547 patients, 348 (63.6%) were never-smokers, 92 (16.8%) had a smoking history of <20 pack-years, and 107 (19.6%) had a smoking history of ≥ 20 pack-years. The baseline characteristics of the groups are summarized in Table 1. The proportions of men, obesity, and alcohol drinkers were higher among the smokers, and there were increasing trends according to increasing number of pack-years. The smokers also had higher prevalences of hypertension and hyperuricaemia.

Correlations between smoking status and pathological changes

The relationships between pack-years of smoking and pathological changes are shown in Figure 4. Luminal stenosis measurements were available in 512 (93.6%) donors who had at least one small–medium artery suitable for evaluation in their biopsy specimen. The prevalence of renal hyalinization increased with increasing number of pack-years (p for trend <0.001). A similar trend was observed for the prevalence of moderate to severe hyalinization (p for trend <0.001). Meanwhile, the prevalence of intimal thickening of small–medium arteries was not related to pack-years (p for trend = 0.83). Although the percentage of global glomerulosclerosis appeared to be higher in the group with ≥ 20 pack-years than in the other groups, significant differences were not observed (p = 0.30; group with ≥ 20 pack-years versus never-smoker group, p for trend = 0.40). The percentage of IF/TA area increased with increasing pack-years (p for trend = 0.02). The relevant data are shown in Supplemental Table 1.

Multivariable logistic analyses for the associations between smoking status and pathological changes

The ORs for the presence of pathological changes according to pack-years of smoking are shown in Table 2. The age- and sex-adjusted ORs for arteriolar hyalinization were clearly higher in the groups with <20 pack-years and ≥ 20 pack-years compared with the never-smoker

group (OR 1.73 [95% CI 1.01–2.94] and OR 2.38 [95% CI 1.43–3.97], respectively; p for trend <0.001). The association remained significant after adjustment for plausible atherosclerotic risk factors (age, sex, BMI, serum total cholesterol, systolic blood pressure, use of anti-hypertensive agents, diabetes mellitus, alcohol drinking habit, eGFR, and serum uric acid). Moreover, the multivariable-adjusted ORs increased linearly with increasing number of pack-years (p for trend <0.001). To further investigate whether smoking duration and number of cigarettes smoked per day were associated with hyalinization, additional analyses were performed (Table 3). The ORs for hyalinization increased significantly with increases in both smoking years and number of cigarettes smoked per day.

The ORs for the prevalence of >10% global glomerulosclerosis according to smoking pack-years are also shown in Table 2. The prevalence of >10% global glomerulosclerosis was significantly higher in the group with ≥ 20 pack-years than in the never-smoker group in the multivariable adjusted model (OR 2.11 [95% CI 1.18–3.79]). Furthermore, the OR increased almost linearly with increasing number of pack-years (p for trend = 0.01). When the associations between prevalence of >10% global glomerulosclerosis and smoking duration and number of cigarettes smoked per day were evaluated, similar associations were observed (Table 4).

Meanwhile, the prevalences of intimal thickening of small–medium arteries and >5% IF/TA were not associated with smoking amount (Table 2).

Association between smoking cessation years and renal arteriolar hyalinization

We further analyzed the association between smoking cessation years and renal arteriolar hyalinization. In this analysis, 14 patients were excluded for missing data on smoking cessation years, and the remaining 533 patients were evaluated. The associations between smoking cessation years and prevalence of arteriolar hyalinization are shown in Supplemental Table 2. In the multivariable adjusted model, the OR for hyalinization versus the never-smoker group

was highest in the group with <10 years since smoking cessation, but also significantly higher in the group with ≥ 10 years since smoking cessation (OR 2.47 [95% CI 1.51–4.04] and OR 2.23 [95% CI 1.03–4.83], respectively).

Subgroup analyses of the presence of renal arteriolar hyalinization according to smoking status

The effect of smoking on renal arteriolar hyalinization was more significant in the group without hyperuricaemia compared with the group with hyperuricaemia (p for interaction = 0.04). The association between smoking pack-years and presence of renal hyalinization appeared to be stronger in younger (<57 years of age, median) patients than in older patients (p for interaction = 0.096). Diabetes mellitus was not investigated in a subgroup analysis due to the small number of patients (N = 21).

Discussion

In this biopsy-based cohort study of kidney donors without CKD, smoking was identified as an independent risk factor for renal arteriolar hyalinization and global glomerulosclerosis. Furthermore, there were dose-dependent relationships between smoking and these pathological changes. The duration of smoking cessation was also related to renal arteriolar hyalinization, and the OR remained higher in the group with ≥ 10 years since smoking cessation compared with the never-smoker group.

The present study revealed significant relationships between smoking pack-years and renal arteriolar hyalinization. Many studies have demonstrated various injurious effects of smoking on arteries. Although the mechanism for development of atherosclerosis in response to smoking remains to be fully elucidated, an increase in oxidative stress has been proposed as an important mechanism. Cigarette smoke contains various oxidants, and circulating levels of oxidative stress markers were found to be increased in smokers (23). Furthermore, peroxynitrite, which is produced from the reaction of chemicals in cigarette smoke, promotes strong cytotoxicity and atherosclerosis via modifying lipids and proteins (24, 25). Smoking also interacts with inflammatory cytokines (26), and accelerates thrombus formation (27). These various mechanisms may be related to arteriosclerotic changes. Renal arteriolar hyalinization, as an arteriosclerotic lesion, leads to ischemic changes in glomeruli. Arteriolar hyalinization is also reported to be associated with loss of renal autoregulation (28). These changes may accelerate glomerulosclerosis. In clinical settings, hyaline arteriosclerosis was found to be associated with poor outcomes in patients with several kidney diseases (29-32). These findings support the relationship between smoking and CKD progression via renal vascular and glomerular damage.

Other important findings of the present study were the dose-response relationships between smoking and renal pathological changes. The ORs for both arteriolar hyalinization

and global glomerulosclerosis became larger with higher pack-years. Moreover, the additional analyses for smoking duration and number of cigarettes smoked per day showed similar associations. These data suggest the presence of a biological gradient that supports causal relationships. Dose-dependent relationships between smoking amount and pathological atherosclerotic changes were previously observed in several other organs in autopsy cases (33-35). Although a dose-dependent relationship was not clearly described in renal arterioles, the renal arteriolar wall thickening was more significant in heavier smokers (12). To the best of our knowledge, this is the first study to demonstrate dose-dependent relationships between smoking and renal pathological arteriosclerotic changes, and these findings may support previous observations for adverse effects of smoking in the incidence of CKD.

In contrast to the present study, a previous renal biopsy study showed that smoking history was significantly associated with intimal hyperplasia of small arteries, but less associated with arteriolar hyalinization (13). The discrepancy between the studies may be partially explained by the difference of target populations. In the previous study, almost all patients had biopsy-proven kidney disease, proteinuria and hypertension. Compared with previous study cohort, our cohort contained relatively healthy individuals, without any proteinuria or primary kidney disease, because they were donors for kidney transplantation operations. Hyalinization is considered an initial lesion for vascular injury (36). Thus, the effects of smoking on renal arteriolar hyalinization may be more apparent in relative healthy individuals. Furthermore, in subgroup analysis of our study, there was a more apparent association between hyalinization and smoking pack-years, especially in younger patients. A difference in renal arteriosclerotic lesions according to age was also observed in immunoglobulin A nephropathy (IgAN) patients (37). In a previous study of IgAN, the prevalence of renal hyalinization was higher in patients with hypertension than in patients without hypertension, and the effect of hypertension on the presence of hyalinization was significant in younger patients (<35 years of age), but not in

older patients. Thus, the vascular responses to arteriosclerotic factors may vary according to age. Further studies are needed to clarify this relationship in more detail.

We also investigated the association between smoking cessation years and renal arteriolar hyalinization. The OR for hyalinization was high in the group with <10 years since smoking cessation, but also remained high in the group with ≥ 10 years since smoking cessation compared with the never-smoker group. The median pack-years were similar between the groups with <10 years and ≥ 10 years since smoking cessation (median 20 [IQR 10–36] and median 20 [IQR 10–30], respectively). From our results, we could not infer whether renal arteriosclerotic lesions would be improved by removal of smoking as a causal factor. However, at least, fewer years since smoking cessation and higher pack-years may be associated with an increase in arteriosclerotic changes. Smoking cessation may reduce the renal vascular burden by decreasing the total smoking amount.

The present study has several limitations. First, it was a single-centre, cross-sectional study. Therefore, we could not determine causal relationships. However, cumulative smoking exposure represents information from the past, and thus causality may be supported. Second, smoking status was evaluated by self-reported questionnaires, and thus reporting bias was unavoidable. Third, the study population comprised kidney transplantation donors who were evaluated for the presence of complications and judged to be able to donate a kidney to their recipients. Therefore, the cohort may be different from a community-based general population. However, the findings strongly suggest that smoking caused renal vascular changes in a cohort without CKD.

In summary, the present study identified smoking as an independent risk factor for subclinical renal injury, arteriolar hyalinization, and global glomerulosclerosis in a cohort without CKD. Furthermore, the prevalences of these pathological changes increased as the cumulative smoking dose increased. These findings support previous reports for a dose-

310 dependent relationship between smoking and progression of CKD. Further studies are required
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315 **Authors' Contributions**

316 Drafted the manuscript: EA. Study design: EA, YM, AT. Data acquisition: EA, YM, KU, AT.
317 Operation performance and data collection: YO. Pathological evaluation: EA, YM. Statistical
318 analysis: EA, YM. Critically revised the manuscript: KU, YM, AT, YO, MN, KM, TN, TK.
319 All authors approved the final version of the manuscript and agreed to be accountable for any
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323 **Conflict of Interest Statement**

324 None declared.

325 **Data Availability Statement**

326 The data underlying this article are available from the corresponding author upon reasonable
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422 **Table 1. Clinical characteristics of the patients by smoking status**

	Never-smokers (N = 348)	<20 pack-years (N = 92)	≥20 pack-years (N = 107)	p for trend
Age (years)	58 (50–65)	50 (38–60)	58 (49–64)	0.99
Male sex (%)	62 (18)	53 (58)	81 (76)	<0.001
Alcohol drinking habit (%)	42 (12)	38 (41)	58 (54)	<0.001
Family history of ESKD (%)	212 (61)	59 (69)	67 (59)	0.49
eGFR (mL/min/1.73 m ²)	83 (77–88)	86 (78–97)	82 (75–88)	0.18
Obesity (%)	70 (20)	20 (22)	32 (30)	0.02
BMI (kg/m ²)	22.3 (20.3–24.2)	22.4 (20.1–24.1)	23.1 (21.0–27.8)	0.04
Hypertension (%)	87 (25)	20 (22)	41 (38)	0.01
Systolic blood pressure (mmHg)	118 (110–130)	117 (108–125)	124 (112–131)	0.07
Diastolic blood pressure (mmHg)	73 (65–80)	72 (63–80)	76 (68–83)	0.09
Use of anti-hypertensive agents (%)	61 (18)	18 (20)	28 (26)	0.03
Use of RAS blockers (%)	32 (9)	8 (9)	17 (16)	0.04
Diabetes mellitus (%)	14 (4)	2 (2)	5 (5)	0.47
Haemoglobin A1c (%)	5.7 (5.5–5.9)	5.6 (5.3–5.8)	5.7 (5.5–5.9)	0.92
Use of oral glucose-lowering agents (%)	10 (3)	2 (2)	3 (3)	0.45
Hypercholesterolaemia (%)	142 (41)	28 (30)	40 (37)	0.30
Serum total cholesterol (mg/dL)	211 (190–237)	206 (186–228)	208 (190–231)	0.80
Use of lipid-lowering agents (%)	54 (16)	5 (5)	11 (10)	0.03
Hyperuricaemia (%)	25 (7)	15 (16)	24 (22)	<0.001
Serum uric acid (mg/dL)	4.5 (3.8–5.3)	5.2 (4.1–6.2)	5.8 (4.6–6.5)	<0.001

423 Data are presented as median (interquartile range) or number (%).

424 ESKD, end-stage kidney disease; BMI, body mass index; RAS, renin angiotensin system; eGFR, estimated glomerular filtration rate.

425 **Table 2. Odds ratios for pathological changes by smoking status**

	Number with finding/total	Unadjusted				Age and sex-adjusted				Multivariable-adjusted*			
		OR	(95% CI)	p	p for trend	OR	(95% CI)	p	p for trend	OR	(95% CI)	p	p for trend
Renal arteriolar hyalinization													
Never-smokers	107/348	1.00	(reference)			1.00	(reference)			1.00	(reference)		
<20 pack-years	39/92	1.66	(1.03, 2.66)	0.04	<0.001	1.73	(1.01, 2.94)	0.04	<0.001	1.76	(1.01, 3.09)	0.047	<0.001
≥20 pack-years	63/107	3.22	(2.06, 5.04)	<0.001		2.38	(1.43, 3.97)	<0.001		2.56	(1.48, 4.44)	<0.001	
Per 20 pack-year increase		1.82	(1.44, 2.30)	<0.001		1.52	(1.18, 1.95)	<0.001		1.50	(1.15, 1.97)	0.001	
>10% global glomerulosclerosis													
Never-smokers	137/348	1.00	(reference)			1.00	(reference)			1.00	(reference)		
<20 pack-years	28/92	0.67	(0.41, 1.10)	0.12	0.19	1.39	(0.77, 2.52)	0.27	0.02	1.50	(0.98, 2.78)	0.20	0.01
≥20 pack-years	53/107	1.51	(0.98, 2.33)	0.06		1.93	(1.11, 3.35)	0.02		2.11	(1.18, 3.79)	0.01	
Per 20 pack-year increase		1.26	(1.04, 1.53)	0.02		1.25	(0.98, 1.58)	0.07		1.24	(0.96, 1.59)	0.09	
Intimal thickening of small–medium arteries [†]													
Never-smokers	119/322	1.00	(reference)			1.00	(reference)			1.00	(reference)		
<20 pack-years	23/89	0.59	(0.35, 1.01)	0.05	0.83	0.87	(0.50, 1.54)	0.64	0.58	0.76	(0.41, 1.42)	0.39	0.46
≥20 pack-years	39/101	1.07	(0.68, 1.70)	0.76		0.77	(0.42, 1.40)	0.39		0.81	(0.45, 1.47)	0.50	
Per 20 pack-year increase		1.12	(0.92, 1.37)	0.24		1.00	(0.80, 1.26)	0.97		0.99	(0.78, 1.25)	0.91	
>5% IF/TA													
Never-smokers	44/348	1.00	(reference)			1.00	(reference)			1.00	(reference)		
<20 pack-years	8/92	0.66	(0.30, 1.45)	0.30	0.22	1.03	(0.46, 1.69)	0.94	0.25	1.03	(0.41, 2.59)	0.95	0.26

≥20 pack-years	20/107	1.59	(0.89, 2.84)	0.12	1.56	(0.75, 3.23)	0.23	1.57	(0.73, 3.38)	0.25
Per 20 pack-year increase		1.26	(1.00, 1.58)	0.049	1.14	(0.88, 1.48)	0.32	1.14	(0.87, 1.48)	0.35

426 OR, odds ratio; CI, confidence interval; IF/TA, interstitial fibrosis and tubular atrophy.

427 *Multivariable adjusted model: adjusted for age, sex, body mass index, serum total cholesterol, systolic blood pressure, use of anti-hypertensive
428 agents, diabetes mellitus, alcohol drinking habit, estimated glomerular filtration rate, and serum uric acid.

429 †A total of 512 patients were evaluated, after excluding 35 patients who lacked any small–medium arteries appropriate for measurement.

430 **Table 3. Odds ratios for the presence of arteriolar hyalinization by smoking years and number of cigarettes smoked per day**

	Number with finding/total	Age and sex-adjusted				Multivariable-adjusted*			
		OR	(95% CI)	p	p for trend	OR	(95% CI)	p	p for trend
According to smoking years									
Never-smokers	107/348	1.00	(reference)			1.00	(reference)		
Smoking ≤20 years	38/86	1.87	(1.05, 3.32)	0.03	0.001	1.90	(1.03, 3.48)	0.04	0.001
Smoking >20 years	64/113	2.15	(1.33, 3.51)	0.002		2.32	(1.39, 3.89)	0.001	
Per 10-year increase		1.25	(1.09, 1.44)	0.001		1.28	(1.11, 1.48)	<0.001	
According to number of cigarettes per day									
Never-smokers	107/348	1.00	(reference)			1.00	(reference)		
Number of cigarettes <20/day	38/84	1.88	(1.11, 3.18)	0.02	0.001	2.07	(1.19, 3.59)	0.01	0.002
Number of cigarettes ≥20/day	64/115	2.22	(1.32, 3.75)	0.003		2.25	(1.29, 3.93)	0.004	
Per 10 cigarette/day increase		1.35	(1.12, 1.62)	<0.001		1.32	(1.09, 1.60)	0.004	

431 OR, odds ratio; CI, confidence interval.

432 *Multivariable adjusted model: adjusted for age, sex, body mass index, serum total cholesterol, systolic blood pressure, use of anti-hypertensive

433 agents, diabetes mellitus, alcohol drinking habit, estimated glomerular filtration rate, and serum uric acid.

434 **Table 4. Odds ratios for the prevalence of >10% global glomerulosclerosis by smoking years and number of cigarettes smoked per day**

	Number with finding/total	Age- and sex-adjusted				Multivariable-adjusted*			
		OR	(95% CI)	p	p for trend	OR	(95% CI)	p	p for trend
According to smoking years									
Never-smokers	137/348	1.00	(reference)			1.00	(reference)		
Smoking ≤20 years	25/86	1.63	(0.85, 3.15)	0.14	0.044	1.74	(0.88, 3.47)	0.11	0.03
Smoking >20 years	56/113	1.68	(1.00, 2.82)	0.048		1.84	(1.07, 3.16)	0.03	
Per 10-year increase		1.15	(1.00 1.33)	0.051		1.18	(1.01, 1.36)	0.04	
According to number of cigarettes per day									
Never-smokers	137/348	1.00	(reference)			1.00	(reference)		
Number of cigarettes <20/day	30/84	1.39	(0.78, 2.46)	0.26	0.02	1.58	(0.87, 2.86)	0.14	0.01
Number of cigarettes ≥20/day	51/115	2.01	(1.13, 3.59)	0.02		2.10	(1.14, 4.84)	0.02	
Per 10 cigarette/day increase		1.20	(0.99, 1.46)	0.055		1.19	(0.97, 1.45)	0.09	

435 OR, odds ratio; CI, confidence interval.

436 *Multivariable adjusted model: adjusted for age, sex, body mass index, serum total cholesterol, systolic blood pressure, use of anti-hypertensive

437 agents, diabetes mellitus, alcohol drinking habit, estimated glomerular filtration rate, and serum uric acid.

438

Legends to figures

Figure 1. Renal arteriolar hyalinization.

Representative images are shown: ah0, no PAS-positive hyaline arteriolar thickening; ah1, mild to moderate PAS-positive hyaline thickening in at least one arteriole; ah2, moderate to severe PAS-positive hyaline thickening in more than one arteriole; ah3, severe PAS-positive hyaline thickening in many arterioles. The following groupings were used for analyses: ah0, no lesions; ah1: mild lesions; ah2 and ah3: moderate to severe lesions. PAS: periodic acid-Schiff.

Figure 2. Intimal thickening of small–medium arteries

Intimal thickening of small–medium arteries was evaluated by the percentage of luminal stenosis. Specifically, the intimal area (between the yellow and red lines) was divided by the area of the intima and lumen (within the yellow line).

Figure 3. Interstitial fibrosis and tubular atrophy (IF/TA)

The cortical area is indicated by the green line, and the IF/TA area is indicated by the blue line. The percentage of IF/TA was defined as the area of IF/TA occupying the total cortical area of the biopsy section.

Figure 4. Pathological changes by smoking status

(a) Proportions of hyalinization severity (no lesions, mild hyalinization, moderate to severe hyalinization). (b) Proportions of intimal thickening severity (no lesions, 1%–49% luminal stenosis, $\geq 50\%$ luminal stenosis). For the analysis, 35 patients were excluded because they lacked any small–medium arteries appropriate for measurement. (c) Percentages of global glomerulosclerosis. (d) Percentages of IF/TA. * $p < 0.01$ versus never-smokers and $^{\dagger}p$ for trend < 0.05 . The p -values for trend were calculated using the Jonckheere–Terpstra test for the percentages of global glomerulosclerosis and IF/TA, and the Cochran–Armitage test for the

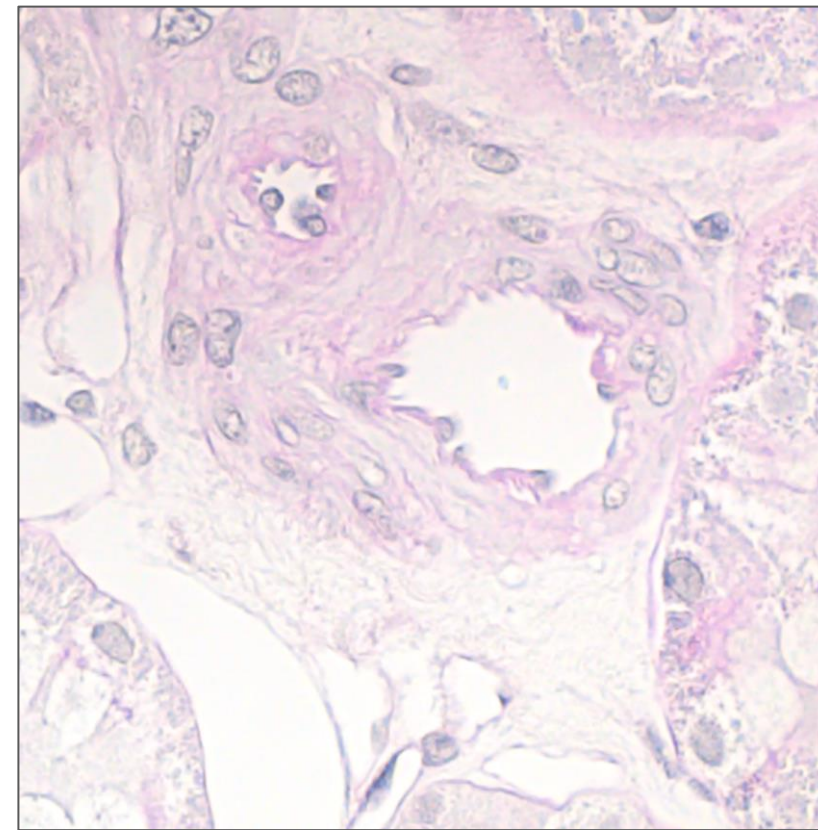
presence of hyalinization and intimal thickening of small–medium arteries. The horizontal line in each box plot represents the median value and the error bars represent the interquartile range.

Figure 5. Subgroup analysis of odds ratios for the presence of hyalinization by smoking pack-years

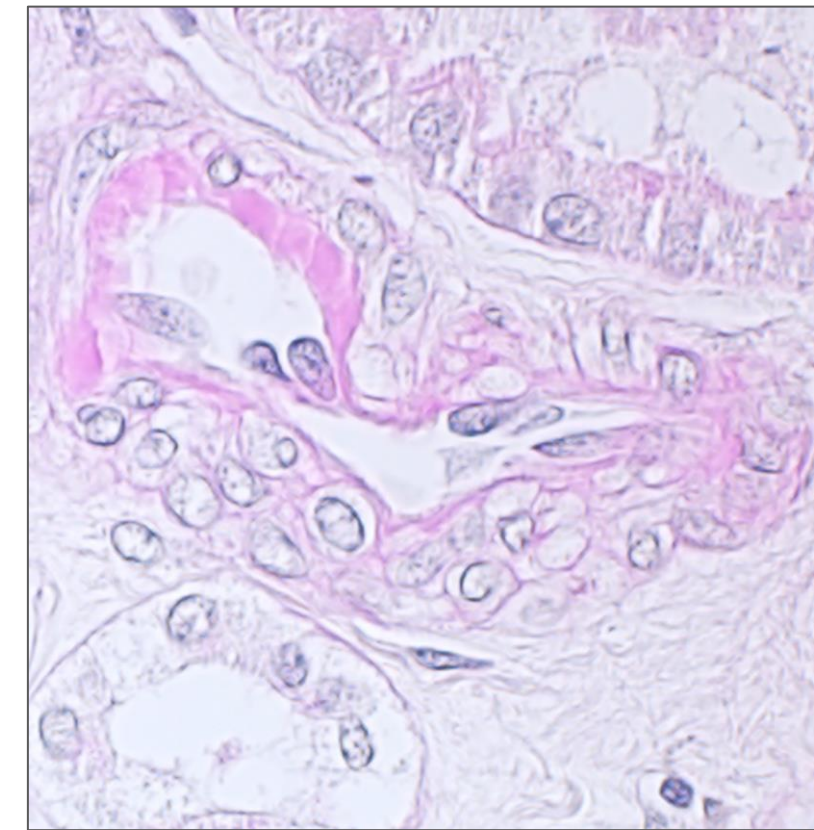
HA, hyalinization; OR, odds ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate.

Figure 1.

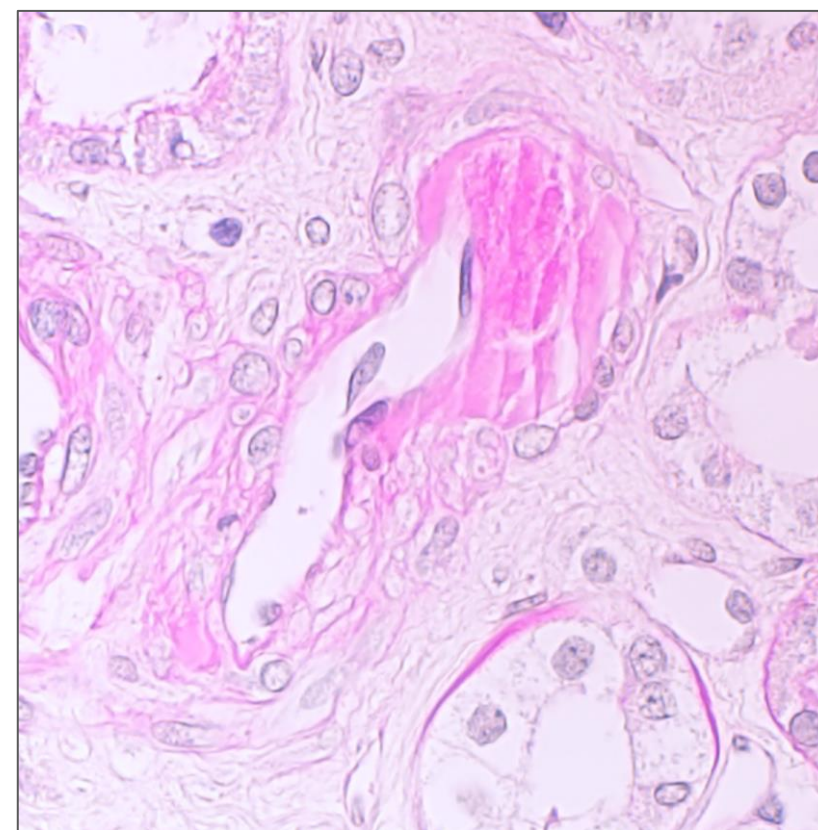
ah0



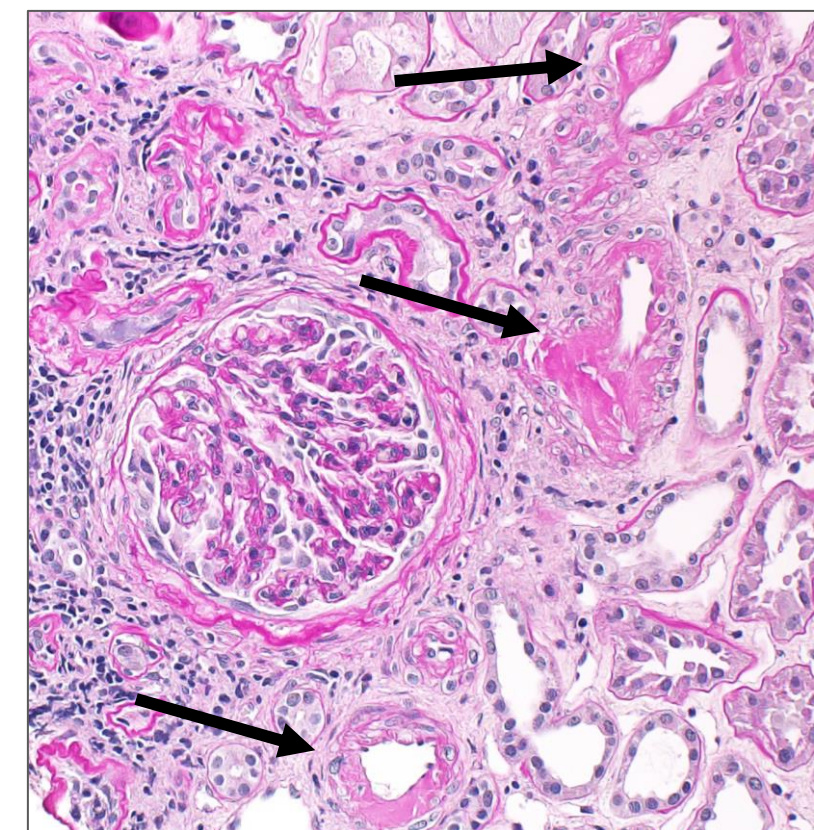
ah1



ah2

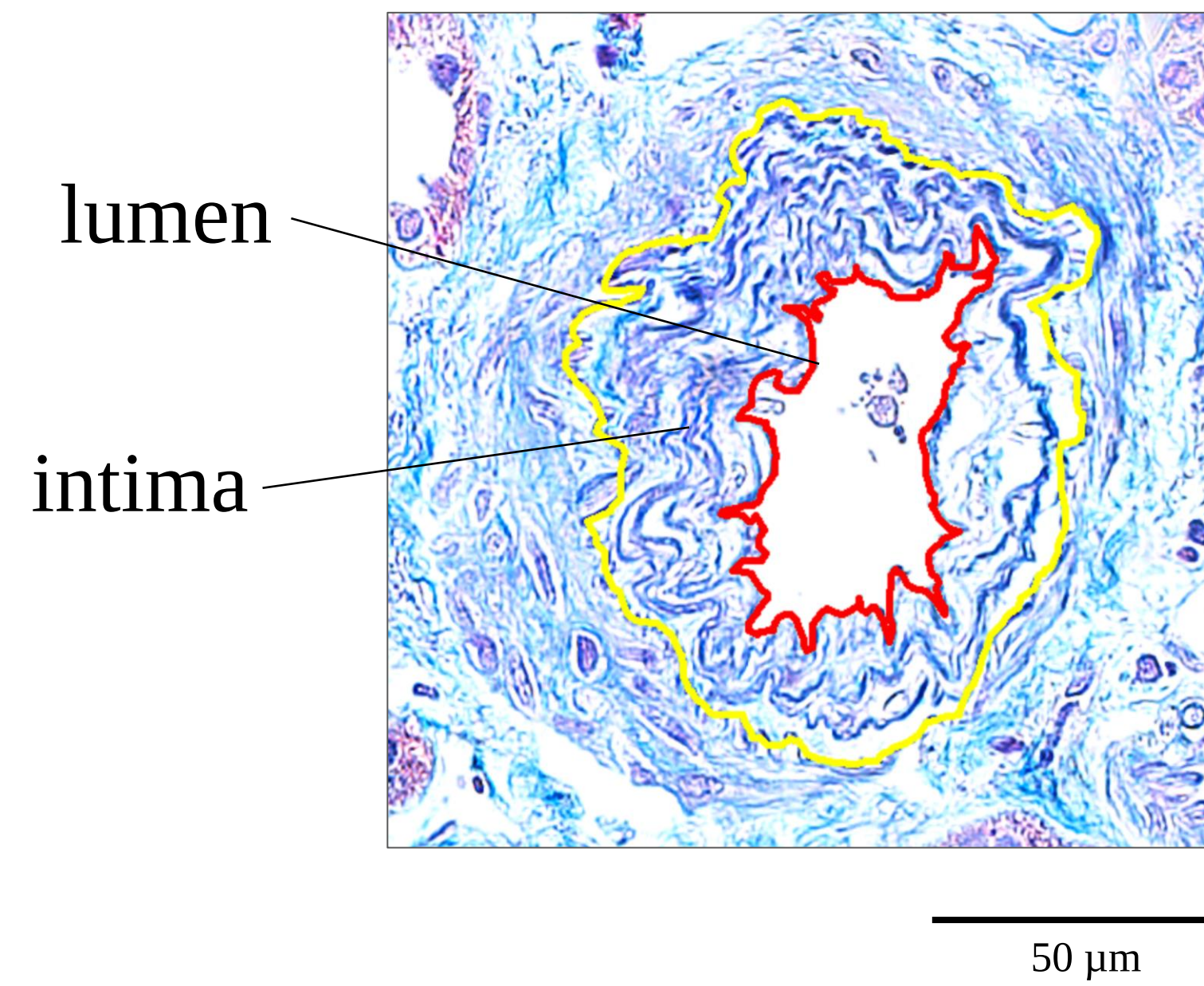


ah3



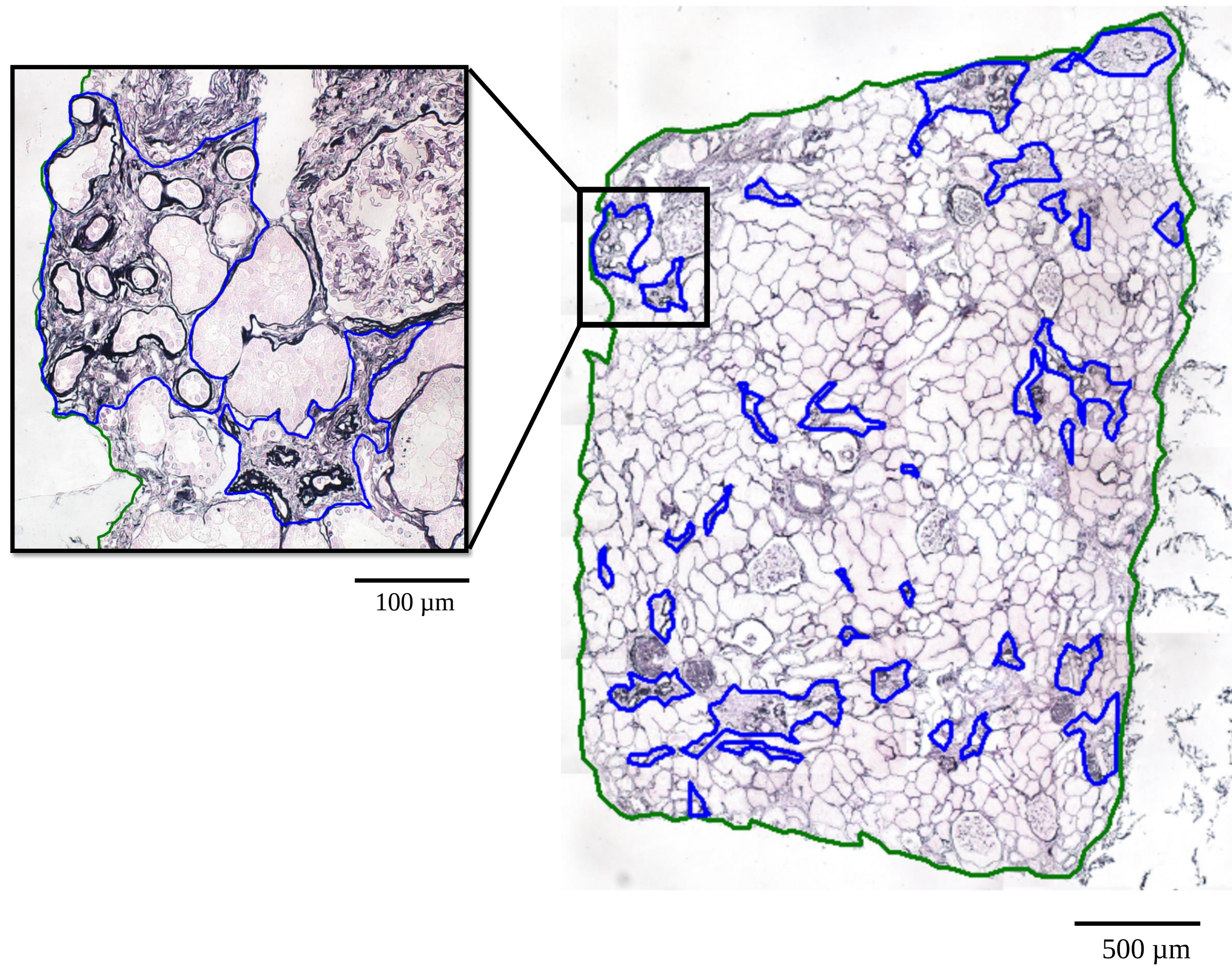
Periodic acid-Schiff stain

Figure 2.



Masson trichrome stain

Figure 3.



Periodic acid-methenamine silver stain

Figure 4.

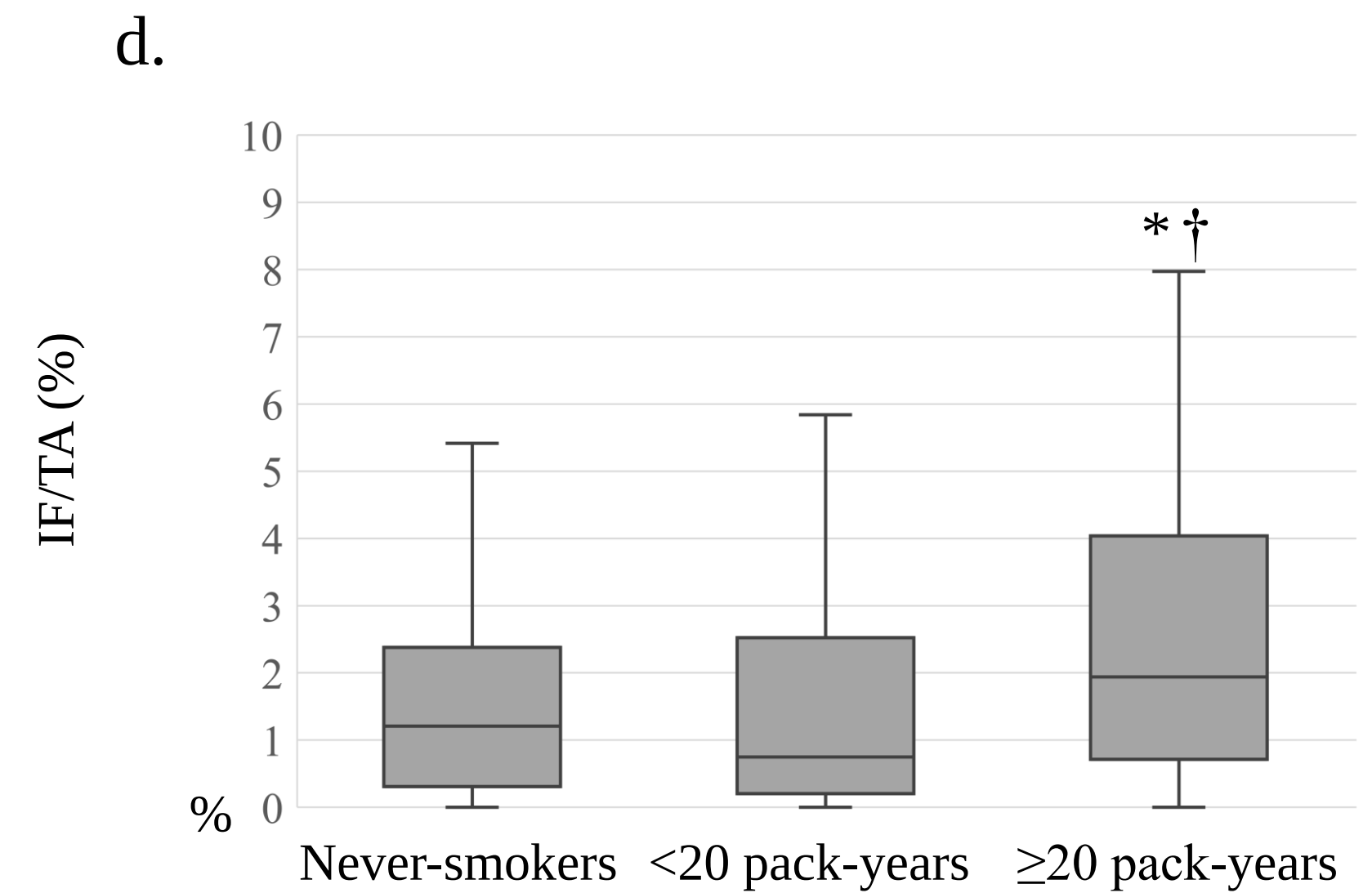
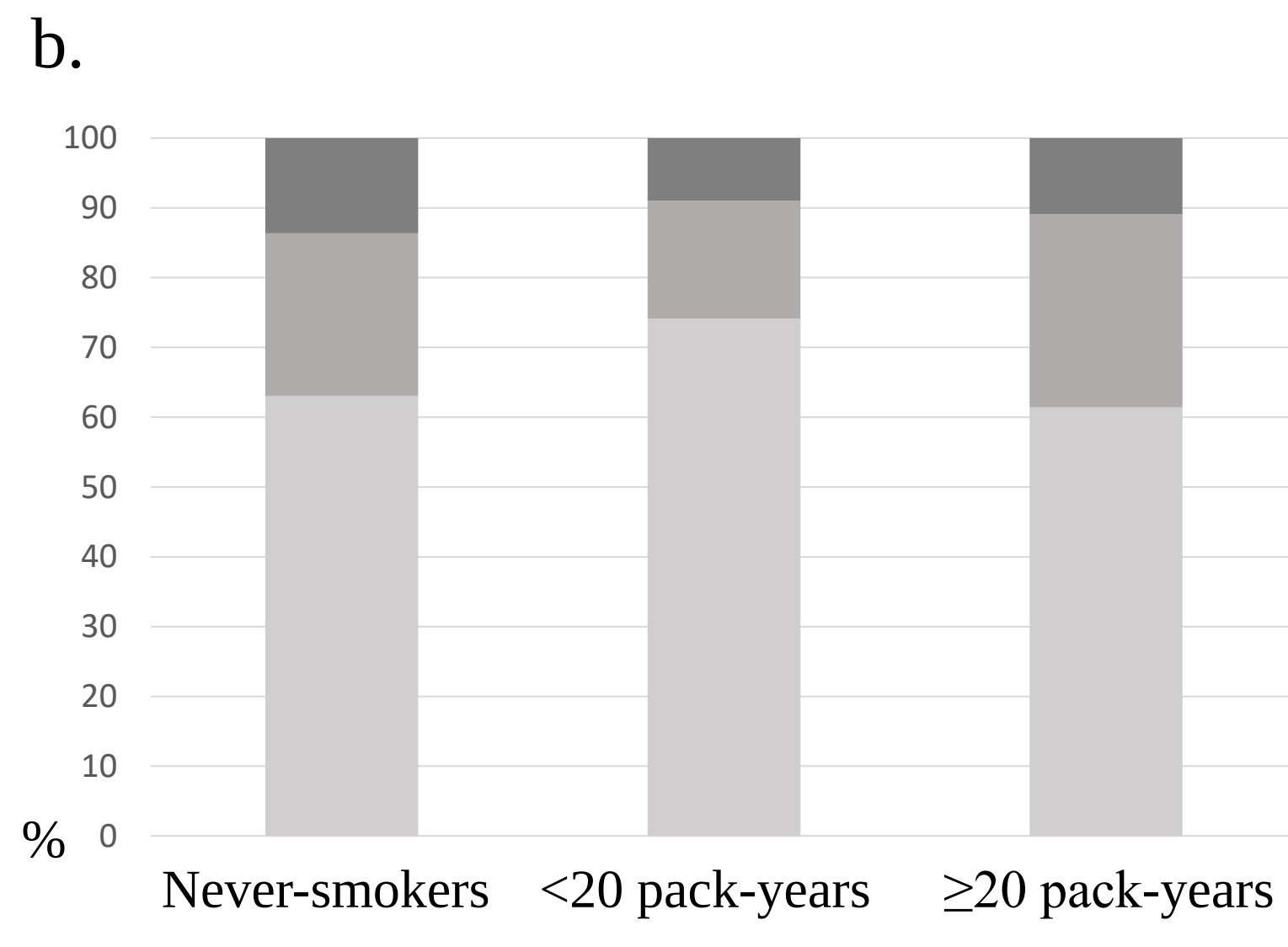
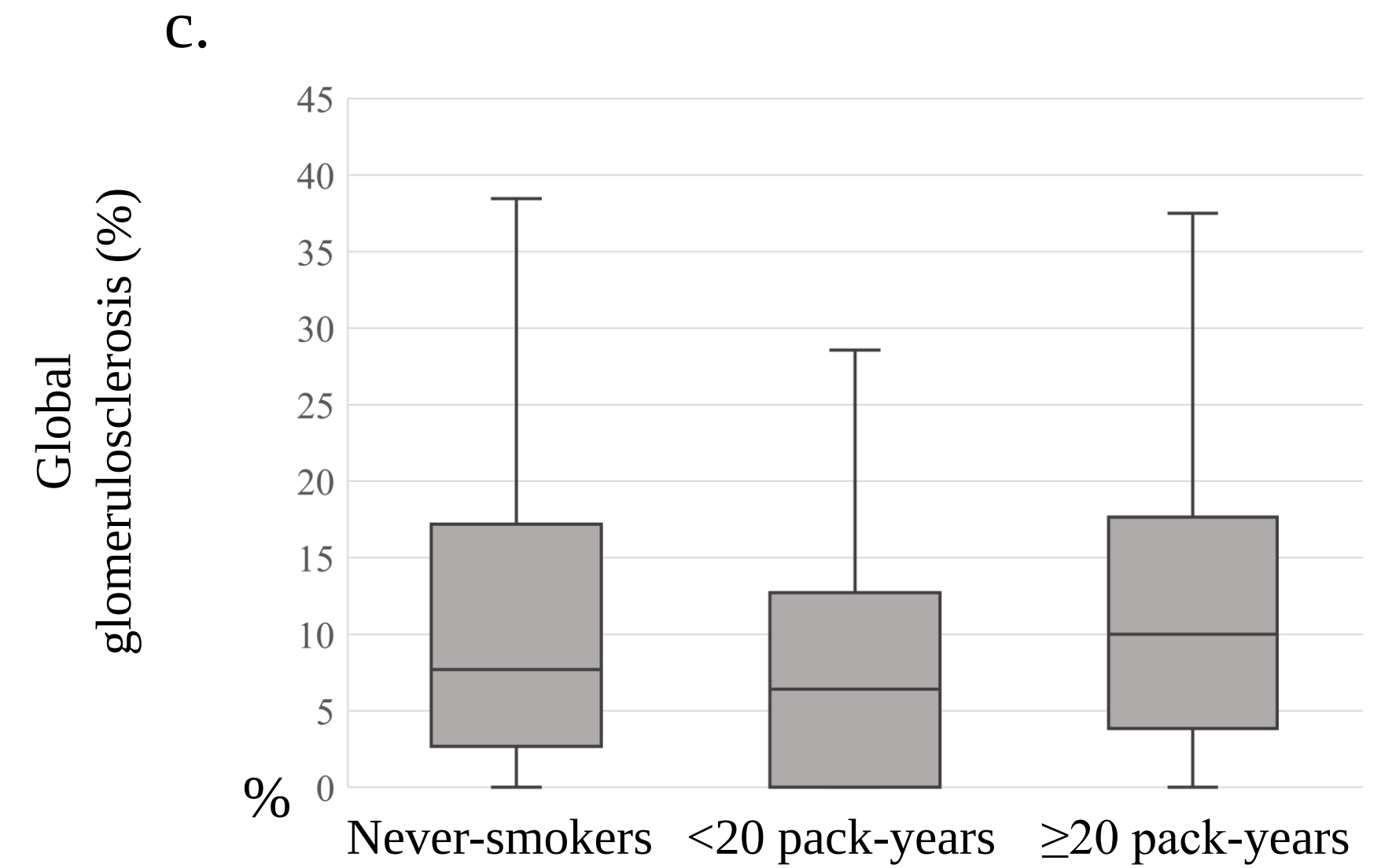
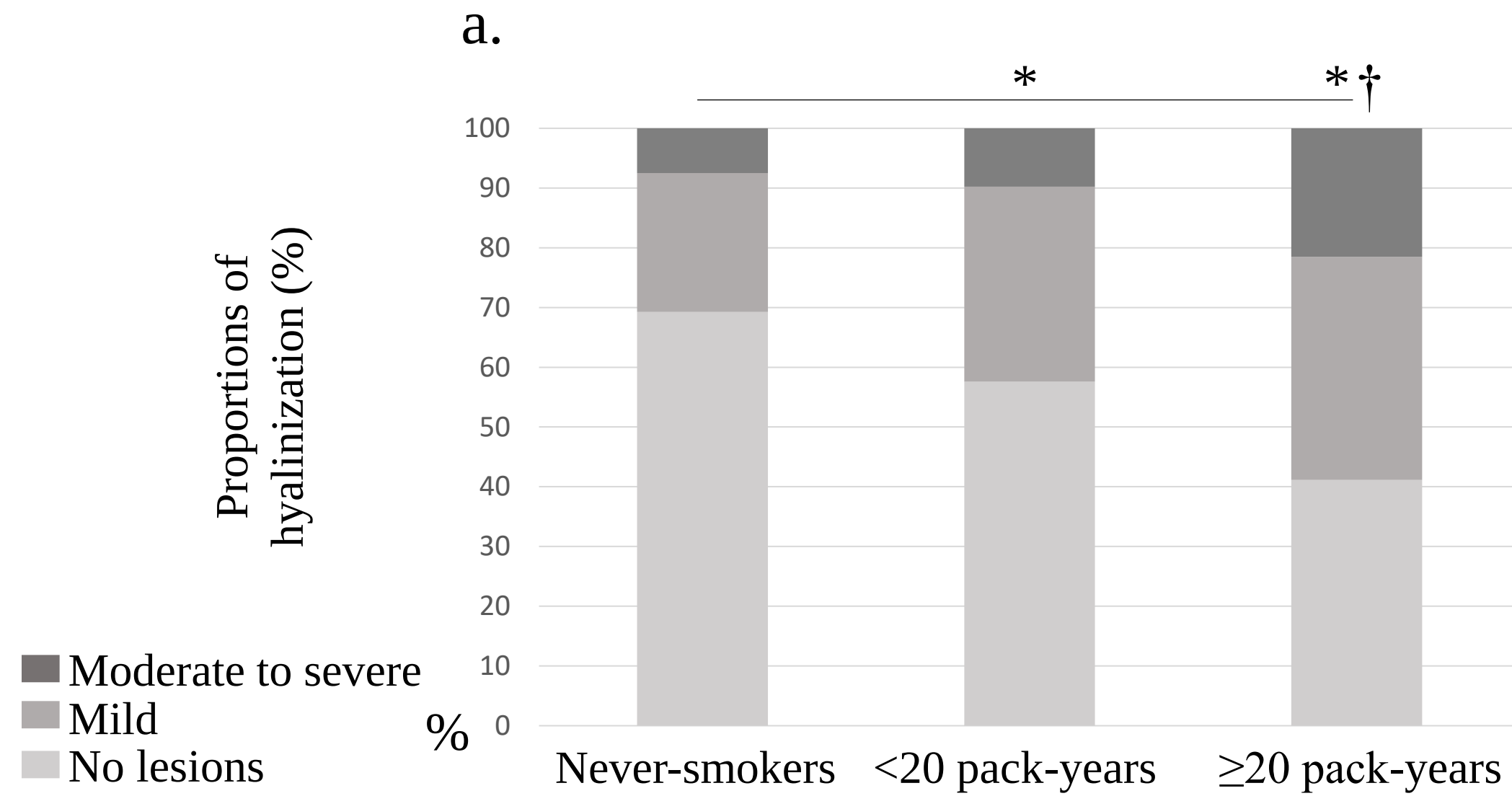


Figure 5.

