

Shadlinskaya S.V.
Azerbaijan Medical University, Baku, Azerbaijan**STRUCTURAL FEATURES OF INTRAHEPATIC BILE DUCTS AND AGE-RELATED MORPHOMETRIC VARIABILITY OF INTRAEPITHELIAL LYMPHOCYTES**

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The insufficient study of the age-related characteristics of lymphoid structures within the intrahepatic biliary tract under normal morphological conditions complicates a comprehensive understanding of the liver's immune defense mechanisms. This research was conducted on 140 cadaveric samples using histological, electron microscopic, and morphometric methods. The reliability of the results was validated through complex biostatistical analysis employing Fisher, Kruskal-Wallis, and Student-Bonferroni criteria. The study provides a detailed description of the hierarchical structure of the biliary excretory system, ranging from bile capillaries formed from the plasmolemma of hepatocytes to the major hepatic ducts. The number of intraepithelial lymphocytes in the intrahepatic bile ducts peaks during early childhood and progressively declines toward senescence. These findings facilitate an objective assessment of donor organ quality in transplantology and allow for the differentiation of age-related immune involution from pathological processes.

Key words: intrahepatic bile ducts, intraepithelial lymphocytes, age-related variability, morphometric indicators, gender differences.

Шадлінська С.В.

СТРУКТУРНІ ОСОБЛИВОСТІ ВНУТРІШНЬОПЕЧІНКОВИХ ЖОВЧНИХ ПРОТОКІВ ТА ВІКОВА МОРФОМЕТРИЧНА МІНЛИВІСТЬ ІНТРАЕПІТЕЛІАЛЬНИХ ЛІМФОЦИТІВ

Недостатнє вивчення вікових особливостей лімфоїдних структур внутрішньопечінкових жовчних шляхів за нормальних морфологічних умов ускладнює всебічне розуміння механізмів імунного захисту печінки. Дослідження було проведено на 140 зразках трупного матеріалу з використанням гістологічних, електронно-мікроскопічних та морфометричних методів. Достовірність результатів підтверджена комплексним біостатистичним аналізом із застосуванням критеріїв Фішера, Краскела-Уолліса та Ст'юдента-Бонферроні. У ході роботи детально описано ієрархічну структуру жовчовивідної системи – від жовчних капілярів, що формуються з плазмолемми гепатоцитів, до великих печінкових протоків. Кількість внутрішньоепітеліальних лімфоцитів у внутрішньопечінкових жовчних протоках досягає піку в період раннього дитинства і прогресивно знижується до похилого віку. Отримані дані дозволяють об'єктивно оцінювати якість донорських органів у трансплантології та диференціювати вікову імунну інволюцію від патологічних процесів.

Ключові слова: внутрішньопечінкові жовчні протоки, інтраепітеліальні лімфоцити, вікова мінливість, морфометричні показники, статеві відмінності.

The widespread implementation of modern diagnostic modalities, particularly high-resolution ultrasonography (USG), has significantly increased the detection rate of gallstone disease (cholecystolithiasis). Recent epidemiological studies indicate that this pathology is recorded in an average of 2 %–15 % of the adult population globally. However, this prevalence varies substantially depending on geographical regions, dietary habits, and lifestyle factors. For instance, while the incidence ranges between 10 %–15 % (reaching up to 20 % in certain populations) in the United States and European countries where a 'Western-style' diet prevails, it remains lower in Asian and African countries, at approximately 2 %–5 %. The development of severe complications such as choledocholithiasis in 10 %–20 % of patients with cholecystolithiasis underscores that the disease is not merely a surgical concern but also a significant socio-economic problem. This situation necessitates the development of modern standards for early diagnosis and the implementation of a differential approach to treatment strategies [8, 11].

Malignant neoplasms of the biliary tract (cholangiocarcinoma and gallbladder cancer) account for approximately 20 % of all hepatobiliary

malignancies, ranking as the second most prevalent oncological pathology of the hepatobiliary system worldwide [13]. The most frequent hepatobiliary tumor is hepatocellular carcinoma, which constitutes approximately 68–75 % of all hepatobiliary neoplasms [10]. In recent decades, a significant increase in the incidence of intrahepatic cholangiocarcinoma (intrahepatic bile duct cancer) has been observed, particularly in Western countries [14].

In diseases of the liver and biliary tract, the interaction between hepatocytes, cholangiocytes, and recruited immune cells – particularly Kupffer cells – determines the clinical outcomes of the pathology. Furthermore, chronic activation of immune cells can trigger not only regenerative repair but also the processes of fibrosis and carcinogenesis [9].

The advancements in modern surgery and therapeutics are directly contingent upon the fundamental study of the microscopic and ultramicroscopic structures of organs [15]. Consequently, there is currently a critical need for a more profound investigation of the liver and biliary tract structures at the microscopic and ultramicroscopic levels. Due to this necessity, these organs remain at the focal point of research for both clinicians (surgeons and therapists) and specialists in fundamental morphology [14].

From a morphological perspective, the lymphoid structures represent one of the least explored components of the liver and biliary tract. In recent years, a series of comprehensive studies dedicated to investigating the lymphoid structures localized within the walls of internal organs have been conducted by representatives of the scientific school of Honored Scientist of Azerbaijan, Professor V.B. Shadlinski (Allakhverdiyev, M.K. (2007); Gasimova, T.M. (2015); Huseynov, B.M. (2011); Huseynova, G.A. (2013); Shadlinskaya, S.V. (2021)) [2]. Despite this, the morphological characteristics of lymphoid structures within the intrahepatic bile ducts under normal conditions remain insufficiently studied to date.

The purpose of the study was to describe the structural features of the intrahepatic bile ducts under normal morphological conditions and to investigate the age-related dynamics of the morphometric indicators of intraepithelial lymphocytes located in their walls.

Materials and methods. The research material consisted of intrahepatic bile ducts obtained from 140 cadavers of both sexes across various stages of postnatal development. Cadavers with identified pathologies of the liver and biliary tract during post-mortem examination were excluded from the study.

Cadaveric materials were obtained between September 2021 and December 2024 from the mortuaries of the Public Legal Entity "Union of Forensic Medical Examination and Pathological Anatomy" of the Ministry of Health of the Republic of Azerbaijan and the Department of Human Anatomy and Medical Terminology of Azerbaijan Medical University. Permission to use the materials was granted by the heads of both institutions.

To safeguard bioethical principles throughout the study, both national and international ethical standards and declarations were observed. The ethical framework of the study was based on the applicable regulatory and legal acts of the Republic of Azerbaijan governing medical practice and bioethics, the World Medical Association Declaration of Helsinki, the international ethical guidelines of the Council for International Organizations of Medical Sciences (CIOMS), and the general principles of bioethics applicable to research involving human biological materials. Approval to conduct the study was obtained from the Ethics Committee of Azerbaijan Medical University (Protocol No. 8, 2021), and all procedures were performed in accordance with accepted ethical standards.

For the microscopic investigation of the intrahepatic bile ducts, histological sections were stained with hematoxylin-eosin and methylene blue. This methodological approach facilitated a comprehensive study of the macro-microscopic architecture of the lymphoid apparatus and the determination of key morphometric parameters, including quantitative indicators of structures, linear dimensions, area, and topographic density (number of structures per 1cm² of the organ wall). For microscopic examination, the cadaveric material was initially fixed in 10 % neutral buffered formalin.

Transverse sections with a thickness of 5–7µm were then prepared from these tissue blocks. To perform hematoxylin-eosin staining, sections were deparaffinized using orthoxylene or toluene. The sections were stained with hematoxylin for 7–10 minutes and rinsed in distilled water for 5 minutes. The sections were kept in 70 % ethanol until they turned brown and were then differentiated in 1 % hydrochloric acid. Subsequently, the sections were washed with distilled water and then with a weak (0.5 %) ammonia solution until they turned blue, followed by staining with an aqueous solution of eosin for 0.5–1 minute. Finally, the sections were dehydrated with 70 % ethanol, cleared with carbol-xylene, and mounted in Canada balsam [6].

For the application of the methylene blue method, the prepared material was smeared in a thin layer onto grease-free, clean slides and air-dried. The preparations were fixed in 96% ethanol for 3 minutes and subsequently dried. The dried specimens were stained with a 1 % alkaline solution of methylene blue for 3-10 minutes, rinsed in water, dried again, and examined under a light microscope [7].

During the electron microscopic examination, fragments from various parts of the liver and bile ducts were collected. The samples were fixed in a solution containing 2 % paraformaldehyde, 2.5 % glutaraldehyde, and 0.1 % picric acid prepared in phosphate buffer (pH 7.4). Subsequently, the tissue specimens were dehydrated and embedded in Araldite-EPON blocks following standard electron microscopy protocols. Semi-thin sections (0.5–1.0µm thick) obtained from these blocks using a Leica EM UC7 ultramicrotome were stained with methylene blue, azure II, and basic fuchsin. These sections were examined under a Primo Star (Zeiss) light microscope, and representative areas were captured using a Canon EOS 650D (Japan) digital camera. Ultra-thin sections (70–100nm thick) obtained from the same blocks were first stained with a 2% uranyl acetate solution, then contrasted with 0.2% pure lead citrate prepared in a 0.1N NaOH solution. The grids were examined under a JEM-1400 (JEOL, Japan) transmission electron microscope at an accelerating voltage of 80–120kV to obtain electron micrographs [3].

The quantitative data obtained from the research were analyzed using modern biostatistical methods. Statistical analysis was performed using Microsoft Excel 2019 and IBM SPSS Statistics 26 software, applying methods of variation and dispersion. For the descriptive analysis of the variation series, the mean (M), standard error of the mean ($\pm m$), and confidence intervals (95% CI (LL) – lower limit, 95% CI (UL) – upper limit) were calculated, along with structural indicators including the median (Me), quartiles (Q1–Q3), and minimum (min) and maximum (max) values. Analysis of variance (ANOVA) was applied for the initial comparison of the series. The significance of differences between grouped indicators based on specific factors was evaluated using the Fisher (F) criterion. The results were further refined using the non-parametric Kruskal-Wallis H-test. The null hypothesis ("0" hypothesis) was

rejected when the statistical significance of the difference was $p < 0.050$. In cases where the null hypothesis was rejected, pairwise comparisons between groups were performed using the Student-Bonferroni criterion to identify specifically between which groups the differences occurred [4].

The final results obtained from the measurements were protocolized, and the collected measurement indicators were compiled into specially prepared statistical cards.

Results of the study. The research results indicate that the intrahepatic biliary tract includes the bile capillaries, as well as the interlobular, segmental, and right and left hepatic ducts. The foundation of the liver lobules is constituted by hepatocyte columns, which collectively form the liver parenchyma. Each plate consists of a series of hepatocytes interconnected by desmosomes. These columns repeatedly anastomose with one another and are oriented radially toward the center of the lobule. In areas where anastomoses are located, a two-row epithelium is formed, and bile capillaries (canals) lacking their own proper walls are situated between these rows (Fig. 1).

The walls of the bile capillaries are formed by the plasmolemma of two adjacent hepatocytes, which create microvilli through invagination. The connection between the plasmolemmas of the

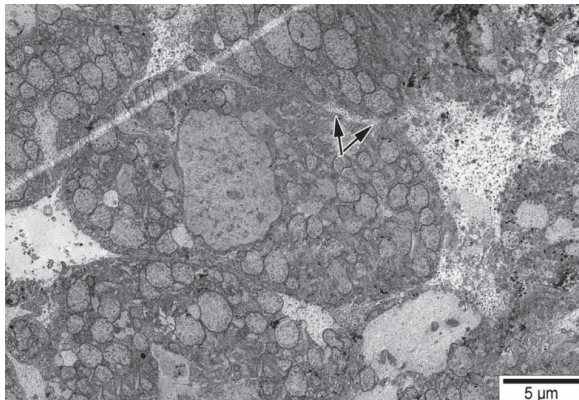


Fig. 1. Bile capillaries (arrows) within a hepatic lobule. Ultrathin section. Electron micrograph. Magnification: $\times 5000$.

The muscular layer is composed of smooth muscle fibers. In the right, left, and common hepatic ducts, it consists of both inner circular and outer longitudinal layers. The outer layer (adventitia) of the intrahepatic bile duct walls is composed of loose connective tissue. Research findings indicate that only intraepithelial lymphocytes are present in the interlobular ducts, while both intraepithelial lymphocytes and diffuse lymphoid tissue are identified in the segmental ducts. In addition to these structures, solitary lymphoid nodules are observed in the right and left hepatic ducts (Fig. 3).

A morphometric method was applied in the study to calculate the quantitative indicators of lymphocytes located within the epithelial layer of the intrahepatic biliary tract (relative to every 100 epithelial cells). The results demonstrate that in the right and left hepatic ducts, the number of intraepithelial lymphocytes reaches its peak during

hepatocytes forming these capillaries is reinforced by a complex of intercellular junctions. Bile capillaries originate blindly in the central region of the liver lobule and are directed toward its periphery. At the periphery of the classic lobule, the bile capillaries open into short canals of Hering, which are lined with squamous or cuboidal epithelium. These canals consist of small cells with oval nuclei arranged in a single row. The canals of Hering are bordered by both hepatocytes and cholangiocytes. In turn, they open into cholangioles that surround the lobules along their circumference. From the cholangioles, interlobular bile ducts are formed, which are lined with simple cuboidal epithelium in smaller ducts and prismatic (columnar) epithelium in larger ones. Bile capillaries discharge into the interlobular ducts, which then merge to form segmental ducts of relatively larger diameter. The fusion of segmental ducts results in the formation of the right and left hepatic ducts, measuring 0.5–2 cm in length. These ducts converge at the porta hepatis to open into the common hepatic duct. Histological examinations demonstrate that the walls of the intrahepatic bile ducts consist of mucous, muscular, and connective tissue layers, which ensure their functionality and protection. The epithelial layer of the mucosa is composed of cuboidal cells in the interlobular and segmental ducts, and columnar cells in the right and left hepatic ducts (Fig. 2).

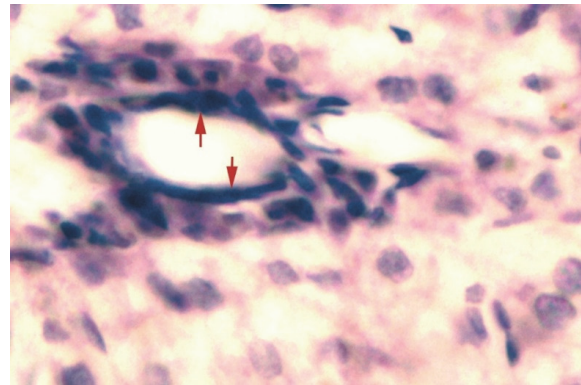


Fig. 2. Cuboidal cells (arrows) in the wall of an interlobular duct. Hematoxylin-eosin staining. Magnification X600.

early childhood, increasing 1.3 times ($P=0.014$) compared to newborns and 1.2 times ($P=0.419$) compared to infants ($M \pm m$: 32.3 ± 2.8 , min: 24, max: 43) (Fig. 4.).

Compared to early childhood, the value of this indicator decreased 1.1 times ($P=0.520$) in the first childhood, 1.1 times ($P=0.379$) in the second childhood, 1.2 times ($P=0.334$) in adolescents, 1.2 times ($P=0.163$) in young adults, 1.3 times ($P=0.127$) in the first and second adulthood, 1.7 times ($P>0.001$) in the elderly, and 2.2 times ($P>0.001$) in old age. In early childhood, the differences between the number of intraepithelial lymphocytes in the right and left hepatic ducts and the indicators for newborns, the elderly, and those in old age were statistically significant; however, no significant differences were observed compared to other age groups. In the segmental duct, the number of intraepithelial lymphocytes reaches its maximum

in early childhood ($M \pm m = 30.5 \pm 1.9$; min-23, max-37), representing a 1.5 times increase ($P=0.005$) compared to newborns and a 1.0 times increase ($P=0.053$) relative to infants. In subsequent age periods, the number of intraepithelial lymphocytes declines. Specifically, compared to early childhood, this indicator decreases 1.1 times ($P=0.392$) in the first childhood, 1.2 times ($P=0.085$) in the second childhood, 1.3 times ($P=0.042$) in adolescents, 1.5 times ($P=0.042$) in young adults, 1.5 times ($P=0.005$) in the first adulthood, 1.6 times ($P=0.006$) in the second adulthood, 1.9 times ($P=0.002$) in the elderly, and 2.4 times ($P>0.001$) in old age. The differences between the intraepithelial lymphocyte counts of segmental ducts in early childhood and those of newborns, adolescents, young adults, the first and second adulthood, the elderly, and those in

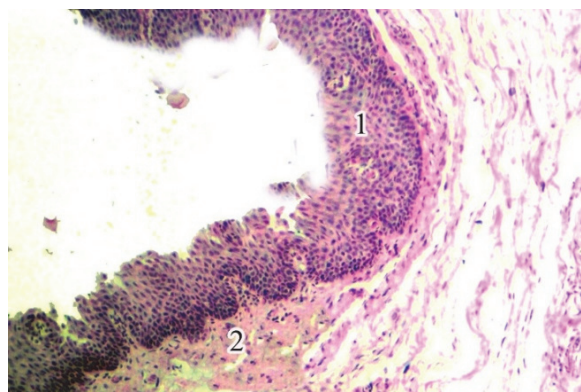


Fig. 3. Lymphoid structures located in the wall of the right hepatic duct. Hematoxylin-eosin staining. Magnification X200. 1. Intraepithelial lymphocytes; 2. Diffuse lymphoid tissue.

Although the quantity of intraepithelial lymphocytes in the interlobular bile ducts during early childhood significantly differs from the newborn, adolescent, first and second adulthood, elderly, and old age groups, this difference remains statistically insignificant ($P>0.05$) when compared to the infancy and first and second childhood periods. The regional characteristics of the distribution of intraepithelial lymphocytes within the intrahepatic bile ducts were analyzed. According to the data obtained, the number of intraepithelial lymphocytes in the right and left hepatic ducts of newborns is 1.2 times higher ($P>0.001$) than in the segmental ducts and 1.5 times higher ($P>0.001$) than in the interlobular ducts. During the infancy period, the number of intraepithelial lymphocytes in the right and left hepatic ducts is 1.2 times higher ($P=0.100$) than in the segmental ducts and 1.3 times higher ($P=0.100$) than in the interlobular ducts. In early childhood, the number of intraepithelial lymphocytes in the right and left hepatic ducts exceeds the values of the segmental and interlobular ducts by 1.1 times ($P=0.136$) and 1.2 times ($P=0.136$), respectively. In the first childhood period, the number of lymphocyte in the right and left hepatic ducts is 1.1 times higher ($P=0.154$) than in the segmental ducts and 1.3 times higher ($P=0.154$) than in the interlobular ducts. During the second childhood, these values are 1.1 times ($P=0.084$) and 1.4 times ($P=0.084$) higher, respectively. Similar ratios were

old age were statistically significant, while differences with other age periods lacked statistical significance. Morphometric analysis revealed a dynamic increase in the quantity of intraepithelial lymphocytes in the interlobular bile ducts. This indicator peaked in early childhood ($M \pm m = 26.3 \pm 1.5$; min-21, max-31), showing a 1.6 times ($P=0.002$) increase compared to newborns and a 1.2 times ($P=0.065$) increase relative to the infancy period. Subsequently, compared to early childhood, it decreases 1.2 times ($P=0.201$) in the first childhood, 1.3 times ($P=0.054$) in the second childhood, 1.4 times ($P=0.019$) in adolescents and young adults, 1.6 times ($P=0.006$) in the first adulthood, 1.7 times ($P=0.004$) in the second adulthood, 2.1 times ($P>0.001$) in the elderly, and 3.0 times ($P>0.001$) in old age.

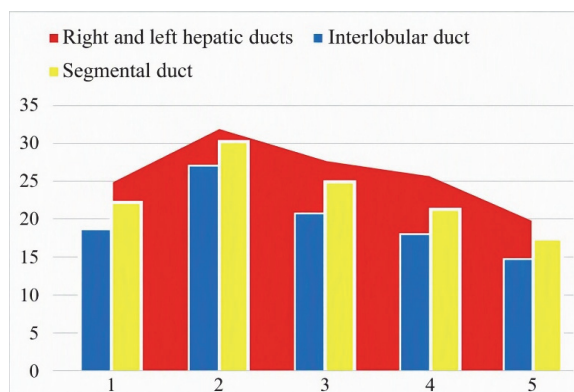


Fig. 4. The number of intraepithelial lymphocytes in the intrahepatic bile ducts in people of different ages: 1. Newborns; 2. Early childhood; 3. Adolescence; 4. 1st adulthood; 5. Old age.

observed in adolescents, with numbers being 1.1 times ($P=0.084$) and 1.4 times ($P=0.084$) higher than those in the segmental and interlobular ducts. In young adults, the number of intraepithelial lymphocytes in the right and left hepatic ducts is 1.1 times higher ($P=0.024$) than in the segmental ducts and 1.4 times higher ($P=0.024$) than in the interlobular ducts. During the first adulthood, these indicators are 1.1 times ($P=0.020$) and 1.5 times ($P=0.020$) higher, respectively. In the second adulthood, the numbers in the right and left hepatic ducts exceed those of the segmental ducts by 1.1 times ($P=0.049$) and interlobular ducts by 1.6 times ($P=0.049$). In the elderly period, the number of intraepithelial lymphocytes in the right and left hepatic ducts is 1.1 times higher ($P=0.005$) than in the segmental ducts and 1.3 times higher ($P=0.005$) than in the interlobular ducts. In the senile period, these indicators are 1.1 times ($P>0.001$) and 1.4 times ($P>0.001$) higher, respectively.

In conclusion, the findings from the morphological study of intrahepatic bile ducts indicate that the intrahepatic biliary excretory pathways originate from the bile canaliculi. These canaliculi initiate at the center of the hepatic lobule, with their walls formed by the plasmalemmal domains of hepatocytes. At the periphery of the lobule, the bile canaliculi drain into the short canals of Hering, which subsequently open into cholangioles. The interlobular bile ducts emerge from

the cholangioles and coalesce to form segmental ducts, which ultimately drain into the right and left hepatic ducts. Unlike the bile canaliculi, the walls of other intrahepatic bile ducts are composed of mucosal, muscular, and connective tissue layers.

Discussion. Trefts E. et al. (2017) conducted a systematic investigation into the normal microanatomy of the liver and concluded that, since the walls of the bile canaliculi are composed of hepatocyte membrane structures, any alterations in this region - such as inflammation, degeneration, or toxic effects - result in the disruption of bile flow and irregular dilation of the canaliculi. Consequently, these factors play a critical role in the pathogenesis of cholestasis and biliary stasis in clinical practice [12].

Until recently, debates have persisted among

pathomorphologists and hepatologists regarding the developmental mechanisms and biological role of the ductular reaction (DR) occurring in chronic liver disease. In the majority of patients with chronic hepatitis, reactive cell chains are observed at the periphery of the hepatic lobule, within the canals of Hering, in the fibrous septa of the liver, and in the subcapsular tissue [5].

Intraepithelial lymphocytes are essential components of the immune system, localized within the mucosa of the gastrointestinal and reproductive tracts. They play a functional role in immunosurveillance and defense against pathogens. The number of these lymphocytes can fluctuate based on various factors, including infections, inflammatory processes, and the overall status of the immune system [1].

Conclusions

1. The morphological study of the intrahepatic bile ducts demonstrates that bile capillaries are formed from the plasmalemma of hepatocytes, opening into the canals of Hering at the periphery, which in turn discharge into the cholangioles and interlobular ducts. The interlobular ducts merge into segmental ducts, which subsequently converge to form the right and left hepatic ducts. The walls of the intrahepatic bile ducts consist of mucosal, muscular, and connective tissue layers.

2. The number of intraepithelial lymphocytes across all intrahepatic bile ducts reaches its peak during early childhood (32.3 ± 2.8), followed by a progressive declining trend in subsequent stages of life. This provides morphometric evidence confirming the variability of the hepatobiliary system's immune defense throughout ontogenesis and its attenuation with advancing age.

3. Regional comparisons indicate that in newborns, the number of lymphocyte in the right and left hepatic ducts is 1.2 times ($P > 0.001$) higher than in the segmental ducts and 1.5 times ($P > 0.001$) higher than in the interlobular ducts. While this ratio exhibits variability across different age periods, the general trend consistently moves from a maximum level in early childhood to a minimum level in senescence.

4. The established age-specific indicators for intraepithelial lymphocytes can serve as a standard criterion in the diagnosis of inflammatory diseases. These indicators may also be utilized as prognostic markers in transplantology to assess the quality of donor organs. Furthermore, the obtained results provide a fundamental basis for the clinical application of new pharmacological agents aimed at strengthening immune defense.

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Conflict of interest. The authors have no conflicts of interest to declare.

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