The prevalence of nonalcoholic liver steatosis in patients with type 2 diabetes mellitus in the Czech Republic

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Aims. Nonalcoholic fatty liver disease (NAFLD) is associated with components of the metabolic syndrome (MS) but the prevalence of NAFLD in the Czech Republic is unknown. The aim of this study was to assess the latter in patients with type 2 diabetes (DM2) and to compare the noninvasive fibrosis scores with ultrasound findings in those patients.

Methods. 180 consecutive patients with DM2 (mean age 64.2±9.3 years, 63% men) were examined for liver biochemistry, MS parameters and had liver ultrasound. MS was diagnosed according to the International Diabetes Federation. The diagnosis of NAFLD was based on liver ultrasound. Other aetiology of liver lesion was ruled out. Additionally, AST/ALT ratio, APRI, NAFLD fibrosis score, FIB4 and BARD scores were calculated.

Results. 93% of patients met the MS criteria, 79% had NAFLD and 13% had ultrasound signs of fibrosis/cirrhosis. NAFLD patients had greater weight (96.9±19.3 vs 84.7±14.7 kg; \( P = 0.003 \)), BMI (32.6±5.2 vs 29.4±5.4 kg/m²; \( P = 0.007 \)), waist circumference (113.8±12.8 vs 107.1±10.3 cm; \( P = 0.033 \)), ALT (0.73±0.57 vs 0.55±0.53 µkat/L, \( P = 0.007 \)) and triglyceridaemia (1.9±1.4 vs 1.4±1 mmol/L; \( P = 0.005 \)) than patients without NAFLD. There were no significant differences in age, sex, cholesterol, fasting glycaemia or glycated haemoglobin. Of calculated scores only the NAFLD fibrosis score revealed significant differences between patients with and without ultrasound signs of fibrosis/cirrhosis (1.027±2.228 vs -0.118±1.402, \( P = 0.026 \)).

Conclusion. Patients with DM2 had in the majority of cases NAFLD which was related to weight, BMI, waist circumference and serum triglycerides. The validity of the liver fibrosis scoring system has to be assessed in those patients in the future.

Key words: non-alcoholic fatty liver disease; steatosis; fibrosis; cirrhosis; metabolic syndrome, type 2 diabetes mellitus

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents a wide range of liver pathology from simple steatosis, to nonalcoholic steatohepatitis (NASH) with typical inflammatory involvement of the liver parenchyma and potential connective tissue deposition up to liver cirrhosis complete with all its complications including hepatocellular carcinoma. Until quite recently, nonalcoholic steatosis was thought to be neither very frequent nor leading to any rather serious liver affections. With a growing body of knowledge revealing a clear connection between NAFLD and civilisation diseases, it now appears that NAFLD is indeed the most frequent cause of abnormal liver tests and, consequently, also of chronic liver diseases in both developed and developing countries. The presence of NAFLD is closely associated with the presence with any of the elements of the metabolic syndrome such as visceral obesity, impaired glucose tolerance, type 2 diabetes mellitus (DM2), arterial hypertension, hypertriglyceridaemia and NAFLD is regarded as part of or, indeed, as a hepatic manifestation of the metabolic syndrome. In this connection, the markedly increased prevalence of NAFLD is likely to be associated with the growing prevalence of obesity and the risk factors involved in it. In the United States, NAFLD affects up to one third of the population, in Europe - up to one quarter. However, only a certain segment of patients with NAFLD face the real threat of contracting liver cirrhosis. Faced with that kind of risk are patients with steatohepatitis (nonalcoholic steatohepatitis - NASH), a condition marked by inflammatory infiltration of the liver tissue, ballooning degeneration of hepatocytes and subsequent fibrosis. The NASH/NAFLD ratio as reported in population studies is in the region of 1:3 (ref.11). Hence, the prevalence of NASH in the general population might reach as much as 5% or more; more down-to-earth estimates put the figure only between 1 to 2% (ref.12). Even that, however, far exceeds, for example, the incidence of chronic hepatitis C. NAFLD has a much higher prevalence (as much as 80%) in groups of patients with diverse high-risk factors such as obesity, DM2 (ref.13), hypertriglyceridaemia and, general-
ly, in those with the metabolic syndrome. The prevalence of NAFLD in particular high-risk groups in the Czech Republic is not known.

The aim of our study was 1) to identify the rate of liver lesion in a group of outpatients with DM2 monitored at the General Teaching Hospital in Prague, 2) to compare serum noninvasive fibrosis scores with ultrasound examination and 3) to assess the applicability of serum noninvasive fibrosis scores in daily routine practice.

METHODS

Under study was a cohort of 180 patients with DM2 treated as outpatients at the 4th Department of Internal Medicine, Charles University 1st Medical Faculty and General Teaching Hospital in Prague (n = 180, age = 64.2±9.3 years, male female sex ratio = 113/67). All of the patients who presented at the above mentioned outpatient unit during the period between January - December 2012 were tested for serum activities of liver enzymes (ALT, AST, GGT, ALP) and concentrations of bilirubin, cholesterol, HDL-cholesterol, triglycerides and uric acid, as well as parameters of diabetes. Basic anthropometric data and ongoing treatment were recorded. 122 patients had ultrasound examination of the liver performed. The ultrasound in all patients was performed by two experienced sonographers (RS and KD). The ultrasound image of the liver was assessed to be normal if the echotexture of the liver was homogenous, with fine level echoes, not hyperechoic compared to the cortex of adjacent right kidney and with adequate visualisation of hepatic vessels. Steatosis was based on an image of bright liver (abnormally high level echoes from the liver parenchyma, hyperechoic compared to adjacent renal cortex), with vessel blurring (impaired visualization of the borders of intrahepatic vessels and narrowing of their lumen) and deep attenuation (evident attenuation of echo penetration into deep portions of the liver). As sonographical signs of liver fibrosis were regarded increased overall echogenicity (but not attenua-
tion), mild irregularities of liver vessels (uneven contours, rarefaction) and slight irregularities of the liver surface. As sonographical signs of liver cirrhosis were regarded coarse echostructure of the liver, uneven liver surface, hypotrophy of the right lobe, hypertrophy of caudate lobe, signs of portal hypertension (portal vein dilation, decrease of portal venous velocity, reversed portal flow, splenomegaly).

The diagnosis of NAFLD was based on ultrasound examination, as stated in AASLD (American Association for the Study of Liver Diseases) guidelines: (a) evidence of hepatic steatosis by imaging (or by histology) and (b) no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders.

In patients without available US, elevated ALT or GGT values (ALT > 0.78 µkat/L, GGT > 0.84 µkat/L) were considered to have suspected liver disease. In the case of patients re-examined during the above period, the highest ALT or GGT values were invariably assessed. Increased ALP and isolated AST activity elevations were not regarded as manifest evidence of NAFLD.

The exclusion of ongoing or recent consumption of significant quantities of alcohol is a fundamental condition to establish the diagnosis of NAFLD. All patients in our study had a negative history of ethanol abuse as indicated by a weekly ethanol consumption of less than 210 g in men and less than 140 g in women. Basically, all examined patients were under regular follow-up at the diabetologic outpatient department and significant consumption of alcohol would be revealed with high probability. The exclusion of significant alcohol intake during the study was based on: 1) The questionnaire used for the detection of alcohol abuse and interviewing the patients (and almost in all cases also a close relative) at repeated follow-up visits and nutritional counselling, 2) the laboratory testing (blood count is a regular laboratory test in these patients and if the macrocytosis was present, additional examinations like CDT, ethylglucuronide in urine, alcohol test in serum were performed. Random testing for alcohol in blood was also implemented. By this testing significant alcohol consumption was excluded sufficiently in all studied patients.

Anamnestic data were employed to eliminate the use of medication that might significantly influence the liver tests (aminosalicylates, paracetamol, glucocorticoids, valproic acid, tamoxifen, amiodarone, warfarin, zidovudine, methotrexate, ampicillin, tetracycline antibiotics, verapamil and diltiazem). Other causes of liver disease were also excluded. Cholestasis was excluded by ultrasound and ALP examination, all patients had performed serology for viral hepatitis (anti HCV, HBsAg, anti HBc and anti HAV). All patients had examined ELFO and if gamaglobulins were elevated, basic screening for autoimmune hepatitis was done. Liver tumours were excluded by ultrasound.

Metabolic syndrome was diagnosed on the basis of the diagnostic criteria of the American Heart Association (AHA) consensus and the International Diabetes Federation (IDF) (ref.15); the presence of the syndrome was also concurrently estimated according to the original IDF (ref.18) and WHO (ref.19) criteria (Table 1). Diabetes compensation was rated in accordance with the glycated haemoglobin value and fasting glucose.

Different scoring systems for the presence of liver fibrosis were calculated based on formulae available in the literature: APRI (AST to Platelet Ratio Index) was calculated as AST(IU/L)/upper AST limit/platelet count (x10⁹/L) x 100 (ref.20), the FIB-4 score according to the formula: age x AST(IU/L)/platelet count (x10⁹/L) x √ALT (IU/L) (ref.21); the NAFLD fibrosis score according to the formula: 1.675 + 0.037 x age (yrs) + 0.094 x BMI (kg/m²) + 1.13 x impaired glucose tolerance or diabetes (yes=1, no=0) + 0.099 x the AST/ALT ratio - 0.013 x platelet count (x10⁹/L) - 0.66 x albumin (g/dl) (ref.20); and the BARD score (BMI, AST/ALT Ratio, Diabetes) as the sum of the following three values: BMI>28 = 1 point, AST/ALT >0.8 = 2 points, diabetes = 1 point23.

Our study was carried out in full accordance with the Helsinki Declaration and approved by the Ethics Committee of the General Teaching Hospital in Prague.

Fig. 1. Frequency of metabolic syndrome in patients with DM2 with and without NAFLD. Metabolic syndrome diagnosed on the basis of AHA/IDF criteria (ref.15), IDF criteria (ref.18) and WHO criteria (ref.19).

<table>
<thead>
<tr>
<th>% of patients with MS</th>
<th>With NAFLD</th>
<th>Without NAFLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>With NAFLD AHA/IDF criteria</td>
<td>98%</td>
<td>81%</td>
</tr>
<tr>
<td>With NAFLD IDF criteria</td>
<td>93%</td>
<td>80%</td>
</tr>
<tr>
<td>With NAFLD WHO criteria</td>
<td>77%</td>
<td>71%</td>
</tr>
</tbody>
</table>
Table 2. Demographic and laboratory parameters in the study cohort.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All patients (n=180)</th>
<th>Patients with NAFLD (n=96)</th>
<th>Patients without NAFLD (n=26)</th>
<th>Patients not examined with USG (n=58)</th>
<th>*P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women (No)</td>
<td>113/67</td>
<td>60/36</td>
<td>15/11</td>
<td>38/20</td>
<td>ns</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.2±9.3</td>
<td>63.4±9.2</td>
<td>66.5±12</td>
<td>64.3±8</td>
<td>ns</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>94.2±18.7</td>
<td>96.9±19.3</td>
<td>84.7±14.7</td>
<td>93.6±18.1</td>
<td>0.003</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.9±5.4</td>
<td>32.6±5.2</td>
<td>29.4±5.4</td>
<td>31.7±5.6</td>
<td>0.007</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>110.8±12.9</td>
<td>113.8±12.8</td>
<td>107.1±10.3</td>
<td>107.4±13</td>
<td>0.033</td>
</tr>
<tr>
<td>Hypertension (% of patients)</td>
<td>82%</td>
<td>81%</td>
<td>77%</td>
<td>83%</td>
<td>ns</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.8±1.4</td>
<td>1.9±1.4</td>
<td>1.4±1.0</td>
<td>1.9±1.5</td>
<td>0.005</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.3±0.3</td>
<td>1.3±0.4</td>
<td>1.3±0.3</td>
<td>1.3±0.3</td>
<td>ns</td>
</tr>
<tr>
<td>Serum glucose fasting (mmol/L)</td>
<td>7.6±3.5</td>
<td>7.8±4.1</td>
<td>7.6±3.5</td>
<td>7.3±2.5</td>
<td>ns</td>
</tr>
<tr>
<td>Glycated Hb (mmol/mol)</td>
<td>53.4±16.4</td>
<td>54.1±17</td>
<td>49.9±12</td>
<td>53.6±17.1</td>
<td>ns</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>10.6±10.4</td>
<td>10.5±10.2</td>
<td>10.9±3.8</td>
<td>10.8±12.8</td>
<td>0.045</td>
</tr>
<tr>
<td>ALT (µkat/L)</td>
<td>0.66±0.49</td>
<td>0.73±0.57</td>
<td>0.55±0.53</td>
<td>0.59±0.28</td>
<td>0.007</td>
</tr>
<tr>
<td>AST (µkat/L)</td>
<td>0.54±0.3</td>
<td>0.6±0.37</td>
<td>0.48±0.21</td>
<td>0.47±0.13</td>
<td>ns</td>
</tr>
<tr>
<td>GGT (µkat/L)</td>
<td>1.02±1.71</td>
<td>1.25±2.14</td>
<td>0.94±1.6</td>
<td>0.69±0.52</td>
<td>0.05</td>
</tr>
<tr>
<td>Uric acid (µmol/L)</td>
<td>322.9±84.8</td>
<td>319±95</td>
<td>332±69</td>
<td>326±72</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Statistical difference between patients with NAFLD and those without NAFLD.

Table 3. Ultrasound findings in patients with DM2.

<table>
<thead>
<tr>
<th>Ultrasound finding</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cirrhosis</td>
<td>5 (4%)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>11 (9%)</td>
</tr>
<tr>
<td>Steatosis</td>
<td>80 (66%)</td>
</tr>
<tr>
<td>Normal finding</td>
<td>26 (21%)</td>
</tr>
<tr>
<td>Total of USG examined patients</td>
<td>122</td>
</tr>
</tbody>
</table>

Statistical methods: The results are presented as means and standard deviation. To estimate the intergroup differences between patients with and without NAFLD, the t-test, ANOVA and the X² test were used. Linear regression was applied for assessing the correlation between the presence of each clinical parameter and the presence of NAFLD. Values of $P \leq 0.05$ were regarded as statistically significant.

RESULTS

Laboratory findings as well as clinical parameters showing the cohort under study are listed in Table 2. Metabolic syndrome rated by IDF criteria was present in 93% of the patients with DM2 or as many as 96% of them if rated by AHA/IDF criteria. Rated according to WHO criteria, the metabolic syndrome affected 76% of the cohort.

To assess the frequency of NAFLD we used a group of patients with available ultrasound examination. The group comprised 122 patients out of the total of 180. Signs of NAFLD were found in 79% of the DM2 patients. Of 58 patients without available ultrasound 10 had a higher ALT (or also GGT) and another 8 had elevated GGT values. These patients were considered to have suspected liver disease.

Steatosis was the most frequent ultrasound finding; 5 patients (4%) had ultrasound signs of liver cirrhosis and 11 patients (9%) had ultrasound signs of fibrosis (see Table 3).

There were no sex or age differences between patients with NAFLD and those without hepatic involvement (the former were little younger but without statistical significance). Nor were there any differences in parameters...
**DISCUSSION**

Data on the high percentage of patients with NAFLD in the high-risk groups (particularly patients with DM2) have been reported in the literature for only a few years and similar data from the Czech Republic are not available. Our study corroborates the high frequency of NAFLD among patients with DM2 and metabolic syndrome. It also shows that the presence of liver disease depends more on particular components of the metabolic syndrome rather than on the compensation of diabetes simultaneously present. As our observations show, nearly all of our patients with hepatic lesion also suffer from metabolic syndrome. Compared with the group without signs of hepatic lesion, the difference in MS frequency is at the borderline of statistical significance.

Like other studies, ours also faced with many adverse factors. To begin with, a liver lesion in NAFLD is difficult to assess accurately. In an ideal situation, it would be appropriate to assess the presence of a hepatic lesion on the basis of liver biopsy. That, however, is impossible in routine practice; moreover, to indicate for liver biopsy, for instance, patients with normal liver test results and merely sonographic evidence of steatosis is unfeasible for ethical reasons. For that reason, liver biopsy will always be reserved for a selected group of patients. Recently, non-invasive techniques have been developed which might replace liver biopsy (different serum fibrosis markers, ARFI type imaging methods or elastography) except that in routine practice these are not often used or readily available. That was why in our study we relied on parameters applicable in routine practice, in particular: serum levels of liver enzymes (ALT and GGT) and liver ultrasound. ALT is an enzyme which rises nonspecifically in liver damage of diverse aetiology (including NAFLD), GGT is an enzyme typically increased not only in alcoholic liver damage but also in NAFLD. While normal ALT (or GGT) values do not rule out the presence of liver fibrosis (a fact known from observations of patients with hepatitis C and NAFLD) (ref.23), no other laboratory parameter can be used in routine practice. Ultrasound investigation was not available in the entire cohort which is why the overall percentage of patients without NAFLD may not be accurate.

Our study was intended to assess the frequency of hepatic lesion in patients with DM2. Rather than a sample of the general population, these were a highly selected group of patients with diabetes and most also with metabolic syndrome. Data concerning steatotic liver involvement in the general population are not currently available, however, a relatively high incidence can be presumed with

<table>
<thead>
<tr>
<th>Parameter</th>
<th>USG fibrosis (n=16)</th>
<th>USG without fibrosis (n=80)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST/ALT (ref.23)</td>
<td>1.321±1.409</td>
<td>0.944±0.340</td>
<td>0.375</td>
</tr>
<tr>
<td>APRI (ref.29)</td>
<td>0.683±0.867</td>
<td>0.347±0.202</td>
<td>0.345</td>
</tr>
<tr>
<td>NAFLD score (ref.23)</td>
<td>1.027±2.228</td>
<td>-0.118±1.402</td>
<td>0.026</td>
</tr>
<tr>
<td>FIB 4 (ref.23)</td>
<td>3.291±4.145</td>
<td>1.527±0.631</td>
<td>0.101</td>
</tr>
<tr>
<td>BARD (ref.23)</td>
<td>2.938±0.998</td>
<td>2.868±1.070</td>
<td>0.853</td>
</tr>
</tbody>
</table>

*APRI (AST to Platelet Ratio Index) was calculated as AST(IU/L)/upper AST limit/platelet count (x109/L) x 100 (ref.20), the FIB-4 score according to the formula: age x AST(IU/L)/platelet count (x109/L) x √ALT (IU/L) (ref.21); the NAFLD fibrosis score according to the formula: -1.675 + 0.037 x age (yrs) + 0.094 x BMI (kg/m²) + 1.13 x impaired glucose tolerance or diabetes (yes=1, no=0) + 0.099 x the AST/ALT ratio - 0.013 x platelet count (x10⁹/L) - 0.66 x albumin (g/dl) (ref.22); and the BARD score (BMI, AST/ALT Ratio, Diabetes) as the sum of the following three values: BMI>28 = 1 point, AST/ALT >0.8 = 2 points, diabetes = 1 point23.
regard to the growing prevalence of overweight and obesity.

Then there is the development (or natural course) of NAFLD. Most observations are focused on one-off evaluation or short-term course. A recently published study rated the prevalence and 7-year development of NAFLD in a cohort of more than 200 “healthy individuals” with no previous history of liver disease. Just under one third of them had sonographic signs of NAFLD upon enrollment in the study. Over a period of 7 years, another 20% developed NAFLD while, on the other hand, about one third of the patients with signs of NAFLD on admission experienced complete regression. Weight reduction was an outright factor responsible for the regression. However, any rating of the story of patients with NAFLD ought to take into account also the chances of spontaneous regression of the disease.

Abnormality alone in liver tests or in sonographic scanning is mostly unable to tell whether a patient has simple steatosis or steatohepatitis (NASH) with the risk of liver cirrhosis development. A distinction of that kind is conceivable with liver biopsy or probably with some of the non-invasive methods such as tests for fragments of cytokeratin 18 (ref.30). The actual uses of non-invasive parameters in these connections will have to be assessed in prospective studies. The presence of fibrosis is another factor decisive for the development of hepatic lesion.

While liver biopsy is still an indispensable tool for liver fibrosis evaluation, sources in the literature describe many non-invasive parameters useful for determining patients with advanced fibrosis from those who have no such affection30-23. However these non-invasive parameters are usually validated for discrimination of different stages of fibrosis in well defined patients with histologically proven NAFLD/NASH and not as a screening test in a risk population. In our own cohort, we tried to apply some of the fibrosis scoring systems which make use of routine laboratory tests and clinical data and which are readily available in the literature. Quite probably these fibrosis scoring systems can be put to use in patients with unambiguously defined NASH/NAFLD; however, their pathognomonic value in this clinical setting is not determined. In a population such as our cohort of patients with DM2 and metabolic syndrome only NAFLD fibrosis score correlated with ultrasound finding, but it showed also frequently positivity in patients without sings of fibrosis on ultrasound. If this was real “false positivity” of the NAFLD fibrosis score, or suspiciously low sensitivity of ultrasound in the detection of fibrosis remains unclear without liver biopsy. In the original NAFLD fibrosis score study by Angulo et al.22, 90 % of NAFLD patients with values of score above 0.676 had histologically verified liver fibrosis. Based on those findings it seems more likely, that standard ultrasound is less sensitive in the detection of fibrosis in our patients. Nevertheless the original study was not validated for use in unsorted DM II patients as a diagnostic criterion of NAFLD and it will be necessary to assess the role of scoring systems in prospective reassessment of liver disease in diabetic patients. In any case this fact emphasizes the need for subsequent management of diabetic patients with fibrosis score (or other scoring systems) positivity. The work-up could embrace the indication to transient elastography or ARFI before considering liver biopsy.

Identification of NAFLD risk factors and their un-delayed control can not only reduce the prevalence of NAFLD but also the extrahepatic diseases associated with this syndrome. For example, the presence of NAFLD is known to carry an increased risk of coronary heart disease (CHD), acute myocardial infarction and association with higher mortality due to CHD in general31.

In conclusion, our observations confirm that a large proportion of patients with high-risk factors (DM2, metabolic syndrome) have NAFLD32. A close connection between elements of the metabolic syndrome and the presence of NAFLD, in particular, overweight (body weight as such, BMI and waist circumference) and the values of serum triglycerides was found. Conversely, the finding that the presence of NAFLD is unrelated to the parameters of diabetes compensation is hardly a very well known fact. As follows from our assessment of a number of biochemical scoring systems for liver fibrosis, those parameters could be examined in unsorted patients with diabetes mellitus, but their clinical relevance has to be established in the future. Based on published cut-off values33, a substantial percentage of diabetic NAFLD patients could be at risk of liver fibrosis. This should be an issue for subsequent studies.

ABBREVIATIONS

AHA, American Heart Association; ARFI, acoustic radiation force impulse; BMI, Body mass index; CHD, Coronary heart disease; DM2, Diabetes mellitus type 2; IDF, International Diabetes Federation; NAFLD, Nonalcoholic fatty liver disease; NASH, Nonalcoholic steatohepatitis; USG, Ultrasonography; WHO, World Health Organisation.

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Authorship contributions: KD, RH, JP: manuscript writing; MZ, TV, AZ, RS, TS, LV, RB: critically revising the content of the manuscript submitted for publication; JP, MZ, AZ, TS, LV: data collection, analysis and interpretation; TV, RS: management of the patients; KD, RH, RB: study design, statistical analysis; RB: final evaluation; KD, RH, JP, MZ, TV, AZ, RS, TS, LV, RB: final approval.

Conflict of interest statement: None declared.
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