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Contrast-Enhanced Ultrasound for the Characterization of Malignant versus Benign Focal Liver Lesions in a Prospective Multicenter Experience – The SRUMB Study

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ABSTRACT

Aim: This study evaluated the accuracy of contrast-enhanced ultrasound (CEUS) for the differential diagnosis of benign vs. malignant focal liver lesions (FLL) in a real-life, multicenter experience.
Methods: This prospective study, including 14 Romanian centers, was performed over a 6 year period (February 2011- April 2017) and included 2062 FLLs assessed by CEUS. Inclusion criteria were: newly diagnosed FLL on B-mode ultrasound, less than three lesions/patient, all FLLs evaluated by CEUS and by a second-line imaging technique (contrast enhanced CT or contrast enhanced MRI) or histology, considered as reference. The trial was registered in clinicaltrials.gov (Identifier NCT01329458).
Results: From the 2062 FLLs included in the study, 57.2% (1179) were malignant and 42.8% (883) were benign. CEUS had 83.9% sensitivity (Se), 97.8% specificity (Sp), 98.1% positive predictive value (PPV), 82.2% negative predictive value (NPV) and a diagnostic accuracy (Ac) of 89.9% for the positive diagnosis of malignant lesions. For the benign lesions, CEUS had 97.8% Se, 83.9% Sp, 82.2% PPV, 98.1% NPV 89.9% Ac. The diagnostic performance of CEUS for hepatocellular carcinoma was 76.6% Se, 98.4% Sp, and 91.2% Ac; for hemangioma: 89.2% Se, 99% Sp, and 96.9% Ac and for metastases: 90.9% Se, 98.4% Sp, and 96.9% Ac.
Conclusions: CEUS proved a high accuracy in differentiating the malignant vs. benign character of a FLL. It can be confidently used as a first line imaging method in daily practice.

Key words: contrast-enhanced ultrasound (CEUS) – focal liver lesions (FLL) – diagnostic accuracy.

Abbreviations: CE-CT: contrast-enhanced computer tomography; CE-MRI: contrast-enhanced magnetic resonance imaging; CEUS: contrast-enhanced ultrasound; EFSUMB: European Federation of Societies for Ultrasound in Medicine and Biology; FLL: focal liver lesion; FNH: focal nodular hyperplasia; HCC: hepatocellular carcinoma; HMG: hemangioma; SRUMB: Romanian Society for Ultrasound and Medicine and Biology; US: ultrasound.

INTRODUCTION

Ultrasound is an inexpensive and reliable method to evaluate abdominal pathologies, and more and more medical specialties use it in daily practice. Starting with radiologists or gastroenterologists and continuing with internal medicine specialists or general practitioners, the "point of care" ultrasound, or clinical ultrasound, is now a standard of care in many areas. The liver is one of the most important organs for ultrasound evaluation, and many focal lesions can be discovered in asymptomatic patients ("incidentalomas") or in patients previously diagnosed with chronic liver disease. For example, liver hemangiomas are present in 1-5% of the general population [1], focal nodular hyperplasias (FNHs) are 10 times less frequent than hemangiomas [2], while focal fatty infiltrations of the liver are not rare.

How to deal with this large number of newly discovered focal liver lesions (FLLs) in daily practice? Immediately after a FLL is found by conventional ultrasound (US), a contrast enhanced ultrasound (CEUS) can be recommended, frequently performed in the same room, using the same machine. This strategy reduces the waiting time and is quite inexpensive in comparison with other cross-sectional methods [3]. For a definitive diagnosis contrast-enhanced computer tomography (CE-CT) or contrast-enhanced magnetic resonance imaging

(CE-MRI) can be also indicated, but this strategy implies delays due to radiological waiting lists and also high costs, plus radiation and/or nephrotoxicity for CE-CT scan.

Many years ago, the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) issued the first guidelines for the use of CEUS [4]. On two occasions, these guidelines were subsequently improved, defining the indications for the second generation ultrasound contrast agents (SonoVue) and the typical CEUS enhancement pattern of different lesions in the liver, and also in extrahepatic organs [5-7]. Unfortunately, in many regions there is still a reticence regarding the use of CEUS for the evaluation of a new FLL. Possible reasons are: insufficient knowledge, insufficient experience with this technique, unavailability of high tech US machines needed to perform CEUS, unavailability of US contrast agent (SonoVue) etc. Considering the price of different imaging techniques, CEUS is the least expensive, using only 1.6-2.4 ml of SonoVue (with a cost around 70 Euros/4.8 ml vial).

Previous large studies from Germany [8], France [9], and Romania [10] demonstrated the good performance of CEUS for the diagnosis of FLLs, confirmed by meta-analyses [11, 12].

The aim of this study was to present the experience of a large prospective multicenter Romanian study, in which CEUS was compared with a reference method: CE-CT, CE-MRI or histology.

METHODS

This prospective study was conducted by the Romanian Society for Ultrasound and Medicine and Biology (SRUMB) and included 14 Romanian centers. It was performed over a period of 6 years (February 2011- April 2017) and included 2062 FLLs assessed by CEUS. The trial was registered in clinicaltrials.gov (Identifier NCT01329458).

Inclusion criteria were: newly diagnosed FLL by conventional US in patients older than 18 years, less than three lesions/patient. All FLLs were evaluated by CEUS and by a second contrast imaging technique (CE-CT, CE-MRI) or histology as reference. Exclusion criteria were: patients with contraindication for CEUS (subjects with acute myocardial infarction, with class III/IV cardiac insufficiency, or with cardiac rhythm disorders, as well as pregnant women); patients diagnosed on conventional ultrasound with simple cysts; patients with previously known FLLs who were evaluated for follow up.

Informed consent for the contrast-enhanced study was obtained from every patient. The study protocol was approved by the local Ethical Committee and was in accordance with the Helsinki Declaration of 1975.

For each lesion included in the study, the following characteristics were documented: patients' demographic data, indication for the CEUS study, the presence of underlying chronic liver diseases or malignancy, the contrast study report, the reference method used for the final diagnosis, as well as the final diagnosis. For each patient, all collected data were registered online via the dedicated website (http://study.umfcv.ro), developed by the University of Medicine and Pharmacy of Craiova for this study.

The CEUS procedure was performed after the FLL was discovered by conventional US. Different US machines were used in different centers, but all contrast studies were performed using SonoVue® (Bracco Spa, Milan, Italy) as a contrast agent, dedicated contrast softwares and low mechanical index. The amount of contrast used was different according to the US machines (1.6 ml or 2.4 ml). The lesion was assessed first in US gray scale; the location, number, size and ultrasound aspect were documented. The conventional B-mode assessment was followed by the contrast study for which SonoVue® was injected via the cubital vein, followed by a 10 ml saline flush. The lesion enhancement pattern was assessed and documented during the arterial (until 30 sec after the contrast bolus), portal (30-120 sec) and late phase (>120 sec). Following the CEUS study, the FLL enhancement pattern was assessed according to the EFSUMB Guidelines [6], and a diagnosis was formulated. The examination was considered conclusive if the FLL had a typical enhancement pattern according to EFSUMB guidelines (for example: hemangioma (HMG): peripheral nodular enhancement in the arterial phase, followed by partial or complete centripetal fill in enhancement in portal and late phase (Fig. 1); focal nodular hyperplasia (FNH): spoke wheel rapid and complete hyperenhancement in the arterial phase followed by iso/hyperenhancement in the

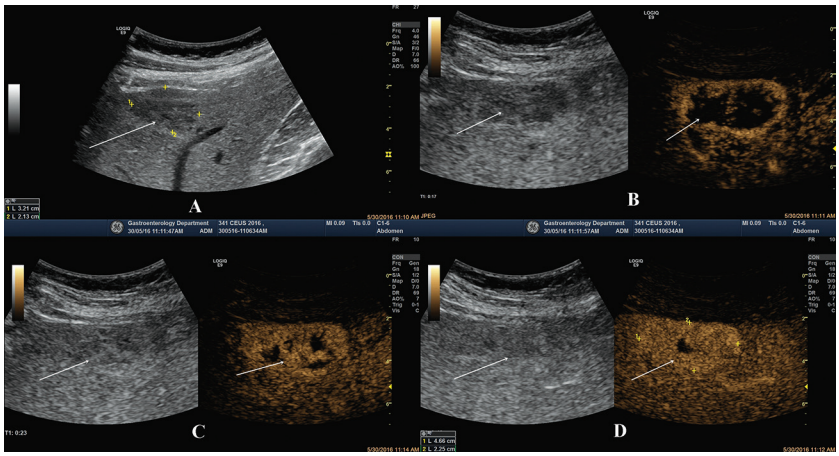

Fig. 1. CEUS study in a hemangioma: B mode (A), peripheral nodular enhancement in the arterial phase (B), followed by partial centripetal fill in enhancement in portal (C) and late phase (D).

portal and late phase (Fig. 2); liver metastases: rim or complete enhancement in the arterial phase followed by rapid wash out in the portal phase with unenhanced lesion in the late phase; hepatocellular carcinoma (HCC): hyperenhancing lesion in the arterial phase, isoenhancing in the portal phase and wash out with hypoenhancing lesion in the late phase (Fig. 3) [6], and inconclusive if the enhancement pattern was not typical.

The CEUS diagnosis was compared with the final diagnosis established based on the reference method (CE-CT, CE-MRI or histology), for example: in typical liver HMG, FNH, focal fatty alterations the definitive diagnosis was based on CE-CT or CE-MRI and on clinical evidence; for typical metastases the diagnosis was based on oncological history and imaging studies, for typical HCCs and regenerative nodules the diagnosis was based on the evidence of underlying chronic liver disease and CE-MRI; in liver abscesses the diagnosis was based on the clinical signs plus imaging studies. In inconclusive cases histology was used for the final diagnosis.

We calculated the sensitivity (Se), specificity (Sp), accuracy (Ac), positive predictive value (PPV) and negative predicted value (NPV) of CEUS expressed as percentages, using the Diagnostic test evaluation tool (Wilson Score method) in OpenEpi version 2.3.1. In case of numerical variables with normal distribution, mean value and standard deviation were calculated, while in case of abnormal distribution, median values and interquartile ranges intervals were presented. Quantitative variables were presented as numbers and percentages.

RESULTS

The mean age of studied patients was 52.4 ± 7.5 years. We detected more FLLs in men (1148, 55.7%) with a mean age of 54.7 ± 7.5. The mean size of FLLs was 4.5 ± 3.3 cm. No contrast agent allergic reactions and no other complications were recorded during this study.

Out of 2062 FLLs examined by CEUS, 1901/2062 (92.2%) had a CE-CT and/or CE-MRI; 470/2062 (22.7%) of them had histology for the final diagnosis, 293/2062 (14.2%) by biopsy and 177/2062 (8.6%) after surgery. The main indications for biopsy were: newly detected liver lesions inconclusive in imaging techniques, new lesions found in patients with an oncological background, new detected lesions in patients with chronic liver diseases.

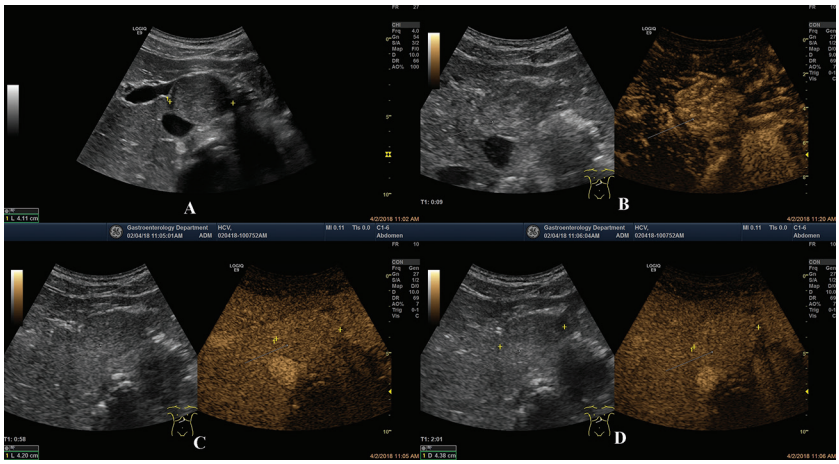

Fig. 2. CEUS study in a focal nodular hyperplasia: B mode (A), spoke wheel rapid and complete hyperenhancement in the arterial phase (B) followed by isoenhancement in the portal (C) and late phase (D).

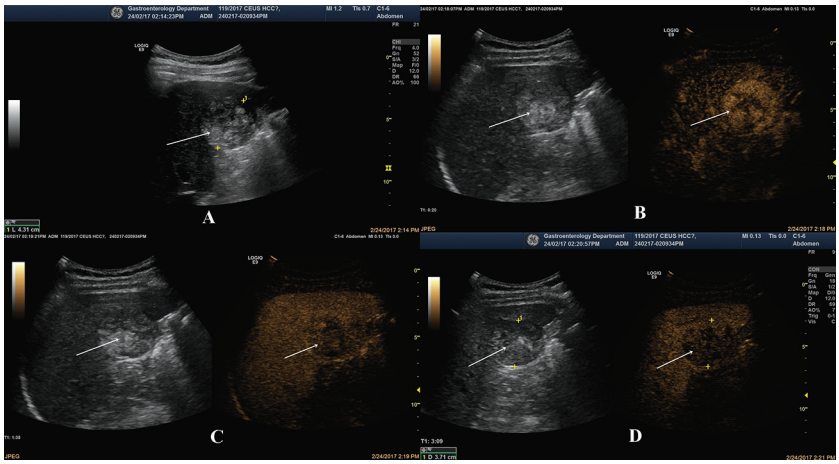

Fig. 3. CEUS study in a hepatocellular carcinoma: B Mode (A), hyperenhancing lesion in the arterial phase (B), hypoenhancement in the portal phase (C) and wash out with hypoenhancing lesion in the late phase (D).

In the sub-group of patients who required biopsy (240, 14,2%), we observed agreement between the initial and the gold-standard result in 240 patients (81.9%) and disagreement in 53 cases (18.1%) respectively.

The analyzed FLL types are detailed in Table I.

Table I. Types of FLL

FLL type	Nr (%)
Hepatocellular carcinomas	685 (33.2%)
Hemangiomas	452 (21.9%)
Metastases	418 (20.3%)
Focal nodular hyperplasias	94 (4.5%)
Regenerative nodules	84 (4.1%)
Focal fatty infiltrations	70 (3.4%)
Cholangiocarcinomas	57 (2.7%)
Abscesses	45 (2.2%)
Complex cysts (inhomogenous cystic lesions)	43 (2.1%)
Other benign lesions*	37(1.8%)
Adenomas	32 (1.6%)
Fatty free areas	26 (1.3%)
Other malignant lesions**	19 (0.9%)

*Other benign lesions: scar area, angiomyolipoma, hamartoma; **Other malignant lesion: lymphoma, hemangiosarcoma, hepatic epithelioid hemangioendothelioma.

The majority of the lesions were detected in patients without chronic hepatopathies (1335, 64.7%). An oncologic history was present in 16.4% of patients. In 47% of cases the lesions were incidental findings and in 1.3% the indication was inconclusive contrast enhanced CT or contrast enhanced MRI. The most frequent lesion was HCC 33.2% (685 cases), followed by HMG 21.9% (452 cases) and liver metastases (20.3%) (418 cases).

From the 2062 FLLs included in the study, 57.2% (1179) were malignant and 42.8% (883) were benign. Considering only the diagnosis of malignancy, CEUS managed a correct differentiation of malignant vs. benign lesions in 88.3% (1820/2062) of the cases. For the lesion-specific diagnosis, CEUS managed a correct diagnosis in 81.4% (1678/2062) of the lesions. As seen in Table II, CEUS had similar Ac for diagnosing benign and malignant lesions (both Ac 89.9%).

Table II. CEUS performance for the benign and malignant class.

	Sensitivity %	Specificity %	Accuracy %	PPV %	NPV %
Benign lesions	97.8	83.9	89.9	82.2	98.1
Malignant lesions	83.9	97.8	89.9	98.1	82.2

CEUS performance for the diagnosis of the most frequent FLLs encountered in daily practice is presented in Table III.

Considering the high incidence of HCCs [13] and the importance of the imaging techniques in the management of this type of lesion, we analyzed CEUS performance separately, according to a threshold diameter of 2 cm (Table IV). The study included 71 HCCs ≤ 2cm and 590 HCCs >2cm.

Table III. CEUS performance for the most frequent FLLs.

	Sensitivity %	Specificity %	Accuracy %	PPV %	NPV %
HCC	76.6	98.4	91.2	96.1	89.4
HMG	89.2	99	96.9	96.4	97
Metastases	90.9	98.4	96.9	93.6	97.7
FNH	84	99.5	98.8	89.7	99.2
Cholangio-carcinoma	61.4	99.3	98.2	71.4	98.9
Abscess	86.6	99.9	99.6	95.1	99.7
Adenoma	56.2	99.9	99.2	90	99.3

HCC: hepatocellular carcinoma; HMG: hemangioma; FNH:ocal nodular hyperplasia

Table IV. CEUS performance for HCCs with a 2 cm threshold.

	Sensitivity %	Specificity %	Accuracy %	PPV %	NPV %
HCC ≤ 2cm	56.3	99.6	91.3	97	90.5
HCC > 2cm	78.9	98.1	91.1	96	89

DISCUSSION

The major advantage of CEUS is that it can be performed immediately after standard ultrasound, when a FLL is discovered. In the future, there might be another application for Point of Care Ultrasound (POCUS). No significant allergic reactions were reported in the published studies [6, 14] and this method can be used in patients with kidney or liver failure. Precaution is needed in subjects with severe heart disorders.

During the first 30 sec after the introduction of contrast bolus (arterial phase), some of the lesions show a typical enhancement pattern (FNHs, HMGs or HCCs). In metastatic lesions, this phase differentiates hyper- from hypo- vascular ones. During the portal phase (30 - 120 sec following the contrast bolus), the vascular pattern will differentiate between benign (which remain enhanced) and malignant lesions (in which wash out occurs, the lesion becoming hypoenhanced as compared with the surrounding parenchyma). An exception to this pattern is seen in HCCs, especially in the well differentiated ones, in which the wash-out is seen later, usually after 3-4 minutes. In the late phase (starting 120 sec. following the contrast bolus until the "bubbles" are destroyed), malignant lesions will continue to wash out. Thus, a hypoenhanced lesion as compared with the surrounding tissue in the portal and/or late phase means malignancy, while hyper- or isoenhanced lesion in these phases signifies a benign lesion. If the examination is made with continuous insonation for the whole 4-5 minutes, the US waves can destroy the "bubbles" and lead to a false aspect of malignancy. For this reason, the current recommendation is to insonate and continuously examine during the arterial phase and after this moment to examine discontinuously [15], to preserve the "bubbles" as much as possible. Detailed technical aspects have been clarified by experts in this field in a recent publication [16].

In some countries such as Japan, South Korea and Norway, another second generation ultrasound contrast is used:

Sonazoid, with the advantage of maintaining the enhancement for more than 20 min. In a recently published meta-analysis, Wu et al. [17] showed that Sonazoid demonstrated a higher diagnostic accuracy in comparison with SonoVue or Levovist (first generation of ultrasound contrast agents).

When a FLL is discovered in a specific clinical context (an apparently healthy subject, or a subject with known cirrhosis or with a history of malignancy), the first question to be answered is if the lesion is benign or malignant. CEUS showed good performance for this purpose (93% Se and 90% Sp) in a large meta-analysis published by Friedrich-Rust, which included 8147 FLLs [11]. In another meta-analysis [12] there were no significant differences between the performance of CEUS and CE-CT/CE-MRI regarding specificity (89% vs. 82% vs 85%) and sensitivity (87% vs. 86% vs 87%) for the diagnosis of malignant liver lesions. The same reliable results were observed in a previous study SRUMB [18]. In this study, in an intention to analyze diagnosis analysis (when all cases were taken into consideration), CEUS was conclusive for benign vs. malignant differentiation in 89.3% of cases. When only conclusive cases were taken into consideration, CEUS had 95.7% Se, 96.4% Sp, 98% PPV, 92.6% NPV and 96% Ac for differentiation between benign vs. malignant lesions.

The results of the performance of the method presented in this study are comparable to those demonstrated by other multicentric studies (Table V) [8, 9].

Table V. CEUS performance for the benign and malignant lesions–comparison with other multicentric studies.

	Sensitivity %	Specificity %	Accuracy %	PPV %	NPV %
DEGUM study [8]	95.8	83.1	90.3	95.4	95.9
STIC study [9]	79.4	88.1	-	-	-
Current study	83.9	97.8	-	98.1	82.2

The performance of CEUS was compared to CE-CT in a study comprising 267 patients, with histological findings available in 158 subjects [19]. CEUS and CE-CT showed similar performance: Ac 90.3 vs. 87.8%, Se 94.0 vs. 90.7%, Sp 83.0 vs. 81.5%, PPV 91.6 vs. 91.5%, NPV 87.5 vs. 80.0%. When CEUS was compared to CE-MRI in 262 patients, there were also no significant differences [20]. The strongest study comparing the performance of CEUS to CE-CT and CE-MRI is the meta-analysis performed by Xie et al, including 25 studies. The pooled estimate Se and Sp calculated for CEUS were 87% and 89%, respectively; for CE-CT they were 86% and 82%, respectively; and finally for CE-MRI, the Se and Sp were 85% and 87%, respectively. The conclusion of this meta-analysis was that there are no significant differences between these three imaging methods.

Regarding the Ac of CEUS for FLL characterization, the last meta-analysis was published in 2018 [21]. It also showed very good performance parameters for CEUS: 0.92 Se (95% CI: 0.91-0.93), and 0.87 Sp (95% CI: 0.86-0.88).

It must be underlined that not all FLLs are as easy or as difficult to diagnose by CEUS. Easy to diagnose are FNHs (where the arterial centrifugal enhancement in the first 10-15 seconds is typical), HMG (with centripetal, progressive, slow, nodular enhancement, sometimes with an unenhancing central thrombotic area) or focal fatty infiltrations (with the same enhancement pattern as the surrounding liver parenchyma in all sequences). Usually, liver metastases show rapid and strong wash-out in the portal phase, so that the final diagnosis can be established in 1-2 minutes. Hepatocellular carcinoma is more difficult to diagnose (76.6% Ac in our cohort), especially well differentiated HCCs, in which the wash-out occurs late or very late (sometimes the contrast microbubbles are destroyed before the wash-out becomes visible). Also, in our study, a low Se was found in small HCCs (≤ 2cm) (56.3%). On the other hand, in the 293 cases where biopsy was performed the main indications were: newly detected FLLs inconclusive in imaging techniques, new lesions found in patients either with oncological background or in patients with chronic liver diseases.

When performing CEUS, the clinical context of the patient should be considered. If the patient is known with liver cirrhosis or if liver elastography performed before CEUS shows high liver stiffness values, a typical arterial enhancement (which appears in approximately 90% of cases) is highly suggestive for HCC, even if the wash-out is present only in approximately 60% of cases [22].

An important aspect of CEUS is its cost/efficiency. Many published studies evaluated this aspect and showed that this method reduces the cost for FLLs' assessment [3, 23, 24, 25]. Data from the DEGUM multicenter study, including 1,349 nodules, showed that the mean saving/patient by using CEUS was 37-101 Euro [23]. In a French multicenter study, CEUS was also the most cost-efficient method, with total savings of 128.5 Euros/nodule [24]. In a Romanian multicenter study, three strategies have been compared: CEUS as first-line imaging method followed by CE-CT or CE-MRI in inconclusive cases; CE-CT in all cases; CT-MRI in all cases [3]. The strategy of CEUS in all cases followed by CE-CT/CE-MRE in inconclusive cases was the most cost effective.

Another advantage of CEUS is that it reduces the time before diagnosis and also the duration of hospitalization.

Despite the fact that our study was a prospective one, it has some weak points. Being a multicenter study, different ultrasound machines were used. Secondly, the experience in CEUS of different centers was not the same, and several operators with different expertise were included in every center. Finally, the operators were mainly clinicians, who were aware of the clinical context of the patient.

CONCLUSION

In this prospective, multicenter, large study, CEUS proved its utility in correctly assessing the malignant vs. benign character of a FLL. Due to its very good performance, it can be confidently used as a first line imaging method in daily practice, and also, as a point-of-care method.

Conflicts of interest: None to declare.

Authors' contribution: I.S., R.B., A.S. conceived and designed of the study. D.L.S., R.S, A.P., M.D., Z.S., C.M., S.I., T.M., C.B., D.N. collected the data. B.T., T.M., R.S., A.P., M.D.: analysed the data. I.S., T.M., R.S.,

M.D., A.P. drafted the article. A.S., V.E. revised the article critically for important intellectual content. I.S., R.S., M.D., A.P., B.T., D.L.S approved the final submitted version.

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