Reproductive Sciences
2018, Vol. 25(4) 498-514

DOI: 10.1177/1933719117750752
journals.sagepub.com/home/rsx
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Transvaginal Elastosonography as an Imaging Technique for Diagnosing Adenomyosis

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Abstract

To test the hypothesis that the lesional stiffness as measured by transvaginal elastosonography (TVESG) correlates with the extent of fibrosis in adenomyotic (AM) lesions, and thus TVESG can be used to diagnose AM, we conducted 2 studies. The first evaluated the relationship, if any, between lesional stiffness and lesional histology in 35 women with histologically confirmed AM in comparison with tissue stiffness in 11 control myometrial (CM) and 8 uterine fibroids (UFs) tissue samples. The second validated the relationship between lesional stiffness and the severity of dysmenorrhea and the amount of menses in AM patients by recruiting 112 patients diagnosed with AM, 67 with UF, and 130 controls. Transvaginal ultrasound and TVESG were both performed. We found that the stiffness of AM lesions was significantly higher than that of UF, which, in turn, was significantly higher than that of CM. Lesional stiffness correlated positively with uterine size and the extent of lesional fibrosis but negatively with E-cadherin and progesterone receptor expression levels. Lesional stiffness also correlated with the severity of dysmenorrhea as well as the amount of menses. Thus, TVESG can improve the diagnostic accuracy for AM, especially in differential diagnosis of AM from UF. The correlation between lesional stiffness and the extent of fibrosis and hormonal receptor expression and the severity of symptomology strongly suggests that TVESG not only can provide an instant assessment of the developmental stage of AM lesions but also may be used to guide the choice of the best treatment modality for the patient.

Keywords

adenomyosis, differential diagnosis, elastography, fibrosis, imaging, ultrasound, uterine fibroids

Introduction

Adenomyosis (AM) is a prevalent, benign gynecological condition characterized by infiltration of endometrial tissues into the myometrium, causing myometrial inflammation and hypertrophy and leading to dysmenorrhea, pelvic pain, abnormal uterine bleeding, and subfertility.[1-6] Its prevalence varies from 14% up to 66%, depending on the patient population and diagnostic procedure and criteria.[5] Adenomyosis often coexists with other gynecological disorders, such as endometriosis and uterine fibroids (UFs),[4] and may share the same pathogenesis as endometriosis.[7] Although the disease is estrogen dependent, progestogenic agents are not very effective, and the use of gonadotropin-releasing hormone (GnRH) agonists is restricted by short duration.[1] Worse, the symptoms quickly recur after discontinuation of GnRH agonists therapy.[8] Although the levonorgestrel-releasing intrauterine system has been reported to have some efficacy,[9] side-effects such as spotting are nonetheless seen in 1 of 3 women, and oligomenorrhea is the most common complaint observed.[9] The definitive treatment for symptomatic AM is hysterectomy,[10] even though the decision of removing the uterus, arguably an iconic symbol of womanhood, can be difficult and even agonizing to make.

Although ~1 of 3 patients with AM are asymptomatic,[1] these may eventually become symptomatic due to the progressive nature of AM lesions, which are essentially wounds undergoing repeated tissue injury and repair (ReTIAR) just like endometriotic lesions.[11-14] In addition, since AM may increase the risk of infertility [15] and preeclampsia,[16] accurate diagnosis becomes a critical first step toward the proper management of AM.

Currently, transvaginal ultrasound (TVUS) and magnetic resonance imaging (MRI) are 2 mainstream imaging techniques used to diagnose AM. Due to its much lower cost as compared with MRI, its ubiquitous, and ease of use, TVUS is now the first-line imaging technique when UF is suspected and

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also the imaging method of choice when AM is suspected.[17] The sensitivity and specificity of TVUS to detect AM are reported to be ~83% and ~85%, respectively, comparable to that of MRI.[18] However, its diagnostic accuracy can be compromised substantially when UF is also present because of the circumscribed nature.[19,20] Even if the diagnosis is accurate, it often provides little, if any, guidance in choosing the best treatment modality.

Thus, there are still ample rooms for improvement in diagnostic accuracy for ultrasonography. In fact, improving the accuracy should also help pave the way for implementation and monitoring of treatment modalities, alternative to more radical treatment such as hysterectomy.

Since AM lesions are also wounds undergoing ReTIAR just like endometriotic lesions, they gradually progress to fibrosis through epithelial–mesenchymal transition (EMT), fibroblast-to-myofibroblast transdifferentiation (FMT), and smooth muscle metaplasia (SMM).[11-14] Hence, the extent of fibrosis can be viewed as the ultimate end result of AM lesions. Incidentally, UF lesions also have excessive extracellular matrix (ECM) deposition.[21] The extent of fibrosis in either AM lesions or fibroids conceivably determines the lesional stiffness or hardness, which could be assessed by palpation through tissue deformation. Hence, lesional stiffness contains information inherently embedded within AM lesions, revealing just how advanced the lesion is. Unfortunately, neither TVUS nor MRI can be used to evaluate the lesional stiffness.

Elastography is a new imaging technology that recently becomes commercially available. It generates images of tissue stiffness, mainly by ultrasound (elastosonography [ESG]). Conceptually, it is akin to the ancient technique of palpation, but it is less subjective, requires no experience, and provides better spatial localization information.[22] Currently, ESG can be roughly categorized into 2 different forms: strain imaging and shear wave imaging.[22] Both methods require mechanical excitation, which is akin to palpation. Depending on the excitation methods, measured physical quantity, and the methods of displaying the measured quantity, ESG can be further divided into different groups.[22] Strain ESG measures the tissue deformation or displacement generated by applying pressure (as an excitation) with a probe on the body surface, whereas shear wave ESG records the propagation of shear waves after excitation. In many commercial ESGs, the tissue stiffness is displayed in false-color image, often side by side with the B-mode image, which greatly facilitates the interpretation of imaging results.

Elastosonography has been proven invaluable to access the extent of fibrosis in liver and to diagnose tumors of the breast and other organs.[23] However, studies on ESG application in gynecology have been scanty, especially in AM and UF. Several published studies reported the use of transvaginal ESG (TVESG) to diagnose AM, and all of them reported that TVESG is a useful tool in diagnosing AM, and in differential diagnosis from UF.[24-27]

In this study, we performed TVESG on patients suspected with AM and/or UF, whose diagnosis was later confirmed histologically. We further performed immunohistochemistry (IHC) and histologic analyses of markers of EMT, FMT, and the extent of fibrosis, along with hormonal receptors, and then evaluated the relationship between these markers and the lesional/tissue stiffness. In addition, we carried out TVESG in a larger sample to validate our findings and also to correlate the stiffness measurement with the severity of symptoms.

Materials and Methods

Patients and Specimens

This study was composed of 2 parts. The first part was designed to evaluate the relationship, if any, between lesional stiffness, as measured by TVESG, and histological parameters (mainly select markers of EMT and FMT and the extent of fibrosis) in women with histologically confirmed AM in comparison with tissue stiffness in control myometrium (CM) and in UF. In addition, we attempted to explore any relationship between tissue stiffness and the severity of symptoms such as dysmenorrhea and the amount of menses. To this end, we recruited 48 premenopausal patients who were initially diagnosed with AM ($n = 37$), or cervical intraepithelial neoplasia (CIN) III ($n = 11$, as controls) by TVUS, symptoms, gynecological examination, and blood biochemistry who were indicated with laparoscopic hysterectomy. After TVESG and TVUS, they all underwent hysterectomy and their conditions were confirmed by histology. Among 37 patients with AM, 2 were later excluded due to suspected perimenopause (one was 56 years old, and the other aged 45 years but had hypertrophic endometrium). As controls, the 11 patients underwent hysterectomy because of CIN-III (100%) but had no myometrial disorder or other gynecologic diseases such as AM or endometriosis. Since 1 challenge in diagnosing AM is confusion with UF, we also recruited 8 patients who were initially diagnosed with UF by TVUS, symptomology, and gynecological examination and after both TVUS and TVESG evaluation underwent hysterectomy, and their UF was histologically confirmed. In all patients, the co-occurrence or the absence of endometriosis was determined by laparoscopy. To minimize confounding, we recruited patients with CM and UF to match the age and the menstrual phase of the AM group as much as we could.

The second part was designed to validate the relationship between lesional stiffness and the severity of dysmenorrhea and the amount of menses in patients with AM, as suggested in the first part of this study. To this end, we recruited 114 premenopausal patients diagnosed with AM, 70 with UF, and 130 controls without AM/UF or endometriosis by TVUS, who visited Shanghai OB & GYN Hospital, Fudan University, from September 2016 to December 2016. Since no hysterectomy was performed and thus no histology in all 3 groups, we acted conservatively by excluding possible AM in the UF and control groups through recruiting those without dysmenorrhea. All these patients showed negative sonographic evidence for ovarian endometriomas. In all 3 groups, the co-presence or absence of endometriosis was determined by laparoscopic evaluation. We later excluded 1 patient from the AM group due to suspected perimenopause (54 years old) or to very young age (23

years) and 3 from the UF group (3 were 53 years or older), leaving 112 patients with AM and 67 patients with UF. Among them, 20 (17.9%) of 112 patients with AM underwent hysterectomy and histological evaluation, but the remaining 92 (82.1%) were diagnosed according to symptomology and conventional TVUS. In patients with UF, 4 (6.0%) of them underwent hysterectomy, while the remaining 63 (94.0%) cases underwent myomectomy. In controls, 10 (17.8%) of them were patients received hysterectomy because of cervical CIN-III (n = 8) or cervical carcinoma in situ (n = 2) but had no myometrial disorder or other gynecological cancers. The remaining 120 (92.3) were healthy volunteers who had no gynecological complaint at all. Among them, 41 were hospital staff, and 79 were women who came to our hospital to take the annual gynecologic examination. None of them had any previous gynecological disorders and symptoms, and the TVUS and TVESG images also show no abnormality. Since in UF and CM groups no hysterectomy was performed and thus no histology, we acted conservatively by excluding symptomatic AM through recruiting those without dysmenorrhea.

All patients' medical records, including clinical symptoms, features, and pathological reports were carefully reviewed and their data retrieved. The demographic information on age, gravidity, parity, length of menstrual cycles, date of the last menstruation, the date of surgery, Verbal Descriptor Scale (none, mild, moderate, or severe) and a 10-cm Visual Analog Scale (VAS) on dysmenorrhea, and the amount of menses (light, if no more than 1 sanitary pad was used in each period; heavy, if more than 3 pads were used; otherwise moderate), as reported previously,[28,29] were collected. For patients with AM, diffuse or focal AM were distinguished by pathological reports for patients who underwent hysterectomy, but this information was not available for those who did not underwent hysterectomy thus had no pathological reports.

For patients who underwent hysterectomy, none of them had taken any anti-platelet, hormonal, oral contraceptive, anti-diabetic, or other medications at least 3 months prior to the surgery. We also reviewed the medical records including clinical features, laboratory results, and pathology reports from hysterectomy as well. For patients with AM recruited to the first part of this study, we collected the AM samples from the patients with AM as well as fibroids tissues from 16 of them who also had co-occurrence with UF. We also collected fibroids tissue samples from 8 patients with UF and myometrial tissue samples from 11 control participants. All collected tissue samples were fixed with 10% formalin and paraffin embedded for IHC analysis and for Masson trichrome staining, as described below.

Written informed consent was sought before the patient was recruited into this study, which was approved by the institutional ethics review board of Shanghai Obstetrics and Gynecology Hospital.

Evaluation of the Sonographic and Elastographic Images

For all recruited participants, the conventional TVUS (B-mode) and TVESG were both performed (all by Y.R. who had 22 years of experience in gynecological sonography in an OB/GYN specialty hospital) on a Hitachi Aloka ARIETTA 70 with an EUP-V53W transvaginal probe (Hitachi, Tokyo, Japan) before surgery (for the first part of the study) to measure the stiffness of AM lesions, UF, or, for controls, just myometrium. This ultrasound system is a real-time strain elastography that detects tissue strain while compressing the surface with the transducer. During acquisition of an elastographic image, the machine quantifies the tissue displacement by tracking ultrasonographic speckles and comparison of changes before and after pressure application (excitation). The amount of change in deformation, as a measure of stiffness, is color coded and is superimposed on the corresponding B-mode image, with red being the softest and blue being the stiffest, and green being the average stiffness.

The uterine size was measured by TVUS. For TVUS imaging, the sonographer (Y.R.) made all her diagnosis based on features as represented in Table 1 from the study by Ferraz et al,[30] which in turn is based on the Morphological Uterus Sonographic Assessment criteria.[31] For the patients with AM and UF, we set a region of interest (ROI) in the typical lesional area of the uterus. The stiffness at the ROI of the uterus was measured during a cycle of compression and decompression with a transvaginal probe. To eliminate possible bias and to maintain consistency, we maintained a probe pressure ranged from 3 to 4 in the indicator for all patients. The Hitachi machine that we used in fact did not give an absolute reading for tissue stiffness. Instead, it only gave liver function index (LFI), which is a built-in stiffness measurement implemented in the Hitachi machines and is displayed automatically after the ROI is positioned. Liver function index is a composite measurement of kurtosis (level of concentration toward the average value of data distribution), degree of strain (a statistical value that indicates the degree of strain away from the symmetrical shape of the histogram), and several quantitative characteristics that indicate the contrast, homogeneity, complexity, uniformity, and direction of tissue texture, initially based on the studies by Tatsumi et al[32,33] and later improved by Fujimoto et al.[34]

In all cases, the LFI for an ROI was measured 3 times, and the mean value was used. The site of the typical lesion in the uterus (such as in the anterior, or posterior uterine wall, or in the fundic uteri), also known as the ROI, was also recorded.

Immunohistochemistry Analysis

Serial 4-mm sections were obtained from each block, with the first resultant slide stained for hematoxylin and eosin to confirm the pathologic diagnosis, and IHC staining of E-cadherin (a marker for EMT), α-smooth muscle actin (α-SMA, a marker for FMT), estrogen receptor β (ER-β), and progesterone receptor (PR) was performed for eutopic and ectopic endometrium in AM, fibroids, and CM tissue samples. Routine deparaffinization and rehydration procedures were performed.

For antigen retrieval, the slides were heated at 98°C in a citric acid buffer (pH 6.0) for a total of 30 minutes and cooled naturally to room temperature. Sections were then incubated with the primary antibody against E-cadherin (1:100; Cell

Table 1. Characteristics of Recruited Control Participants and Patients With Adenomyosis and Uterine Fibroids for the First Part of This Study.

Item	Control (n = 11)	Adenomyosis (n = 35)	Uterine Fibroids (n = 8)	Statistical Significance[a]
Age, years	Mean (SD) = 41.5 (3.8) Median (range) = 41 (34-47)	Mean (SD) = 44.1 (4.3) Median (range) = 44 (34-52)	Mean (SD) = 40.8 (5.5) Median (range) = 42 (33-48)	.09
Menstrual phase				
Proliferative	4 (36.4%)	18 (51.4%)	5 (62.5%)	.52
Secretory	7 (63.6%)	17 (48.6%)	3 (37.5%)	
Parity				
0	0 (0.0%)	3 (8.6%)	1 (12.5%)	.58
1	9 (81.8%)	25 (71.4%)	7 (87.5%)	
≥2	2 (18.2%)	7 (20.0%)	0 (5.0%)	
Severity of dysmenorrhea				
None	11 (100.0%)	4 (11.4%)	8 (100.0%)	1.0×10^{-8}
Mild	0 (0.0%)	3 (8.6%)	0 (0.0%)	
Moderate	0 (0.0%)	10 (28.6%)	0 (0.0%)	
Severe	0 (0.0%)	18 (51.4%)	0 (0.0%)	
Scores of VAS on dysmenorrhea	Mean (SD) = 0.0 (0.0) Median (range) = 0 (0-0)	Mean (SD) = 6.0 (2.8) Median (range) = 7 (0-9)	Mean (SD) = 0 (0) Median (range) = 0 (0-0)	1.7×10^{-7}
Amount of menses				
Light	0 (100.0%)	0 (0.0%)	0 (0.0%)	5.5×10^{-4}
Moderate	10 (0.0%)	11 (31.4%)	6 (75.0%)	
Heavy	1 (0.0%)	24 (68.6%)	2 (25.0%	
Type of adenomyosis				
Focal	NA	7 (20.0%)	NA	NA
Diffused		28 (80.0%)		
Co-occurrence of endometriosis				
No	11 (100.0%)	26 (74.3%)	8 (100.0%)	.30
Yes	0 (0.0%)	9 (25.7%)	0 (0.0%)	
Co-occurrence of uterine fibroids				
No	NA	18 (51.4%)	NA	NA
Yes		17 (48.6%)		
Uterine size, cm^3	Mean (SD) = 67.0 (16.7) Median (range) = 65.3 (48.5-91.1)	Mean (SD) = 354.8 (213.6) Median (range) = 254.3 (55.3-846.3)	Mean (SD) = 239.6 (126.2) Median (range) = 219.5 (33-48)	4.1×10^{-6}

Abbreviations: NA, not applicable; SD, standard deviation; VAS, Visual Analog Scale.
[a]*$P < .05$; **$P < .01$; ***$P < .001$; NS: $P > .05$. Kruskal rank test was used for age and uterine size while for other data Fisher exact test was used.

Signaling Technology [CST], Massachusetts), α-SMA (1:100; Abcam, Cambridge, England), ER-β (1:500; Abcam), or PR (1:100; Abcam) overnight at 4°C. After slides were rinsed, the horse radish peroxidase–labeled secondary anti-rabbit/mouse antibody detection reagent (Shanghai Sun BioTech Company, Shanghai) was incubated at room temperature for 30 minutes. The bound antibody complexes were stained for about 1 to 2 minutes or until appropriate for microscopic examination with diaminobenzidine and then counterstained with hematoxylin (30 seconds) and mounted. The positive staining was evaluated using a semiquantitative scoring system, as reported previously.[35,36] Briefly, the number and intensity of positive cells were counted by Image-Pro Plus 6.0 (Media Cybernetics Inc, Bethesda, Massachusetts). A series of 3 to 5 randomly selected images on several sections were taken to obtain a mean value. Immunohistochemistry parameters assessed in the area detected included (a) integrated optical density (IOD), (b) total stained area (S), and (c) mean optical density (MOD), which is defined as MOD = IOD/S, equivalent to the intensity of stain in all positive cells.

Human invasive breast cancer tissue samples were used as positive controls. For negative controls, human AM tissue samples were incubated with rabbit or mouse serum instead of primary antibodies.

Masson Trichrome Staining

Masson Trichrome staining was used for the detection of collagen fibers in tissues. Tissue sections were deparaffinized in xylene and rehydrated in a graded alcohol series and then were mordant in Bouin solution at 37°C for 2 hours. Bouin solution was made with saturated picric acid 75 mL, 10% formalin solution 25 mL, and acetic acid 5 mL. The tissue sections were stained using Masson Trichrome Staining kit (Baso, Wuhan,

China) following the manufacturer's instructions. The areas of the collagen fiber layer stained in blue were calculated by the Image Pro-Plus 6.0.

Statistical Analysis

The comparison of distributions of continuous variables between or among 2 or more groups was made using the Wilcoxon and Kruskal tests, respectively. Pearson or Spearman rank correlation coefficient was used when evaluating correlations between 2 variables when both variables were continuous or when at least 1 variable was ordinal. To evaluate which factors were associated with the tissue stiffness, multiple linear regression analysis was used. To see which covariables were associated with heavy menses (as opposed to light and moderate menses), a multiple logistic regression analysis was used. Jonckheere-Terpstra test was used to see whether there is trend among different severity groups.

P values of <.05 were considered statistically significant. All computations were made with R version 3.4.2 (www.r-project.org).

Results

The characteristics of patients recruited for the first study are listed in Table 1. As expected, the VAS scores correlated positively with the Verbal Rating Scale (VRS, $r = 0.98$, $P < 2.2 \times 10^{-16}$). Within women with AM, there was no difference in severity of dysmenorrhea between those with or without concurrent endometriosis ($P = .58$) and with or without fibroids ($P = .95$).

Overall, TVESG provided a clear view of the tissue stiffness: the serosal surface of uterus typically showed red-colored images, whereas the endometrium showed reddish to yellowish coloration, and the normal myometrium, as mildly stiff tissues, often showed the mostly greenish and less yellowish coloration (Figure 1A). Fibroids were stiffer than normal myometrium, and their images were mostly shown as green and slightly blue colors, and were surrounded by circling pseudocapsule with red coloration (Figure 1B). The stiffest tissues showing broad areas of blue color were typically seen in severe AM lesions (Figure 1C) and in some focal AM lesions (Figure 1D, the upper left area). In some cases, AM coexisted with UF which showed a typical red-colored pseudocapsule circling around the fibroid (Figure 1D, the middle lower area). Thus, TVESG could easily distinguish AM from fibroids.

In addition, TVESG was found to be superior to TVUS in diagnosing AM when the uterus in question was slightly enlarged ($\sim$5 weeks of gestation). We had 10 cases who complained about dysmenorrhea and had elevated CA-125, but upon conventional B-mode TVUS examination the images showed no signs that were consistent with a typical or spherical enlarged uterus, or the presence of mild but not severe or obvious internal inhomogeneous echo in ROI for diffuse AM or, in the case of focal AM, showed local inhomogeneous echo in the ROI as compared with surrounding myometrium, with no clear boundary to the surrounding myometrium like a pseudocapsule circling around the fibroid. Hence, these patients were suspected with AM but were difficult to be diagnosed with AM by TVUS (Figure 2A).

Under TVESG, however, the ROI of these patients showed higher tissue stiffness than those of normal myometrium (the area pointed by an arrow in Figure 2B), indicative of fibrotic tissues and thus AM lesions. Based upon the symptom and the TVESG, AM in these patients were further confirmed by higher stiffness value under TVESG. For these patients, although the stiffness was higher than those of normal myometrium, they were around 2.6, still lower than the mean lesional stiffness of all recruited patients with AM (suggesting that they may be responsive to hormonal treatment). They were then given treatment with oral contraceptives or progestins, and all were responsive. Therefore, these lesions were diagnosed as AM.

In patients with UF, those whose fibroid nodes histologically diagnosed with hyaline degeneration show even hypoechoic and inhomogeneous echo by TVUS (B-mode) and could be further verified by TVESG which showed significantly lower stiffness than those without (data not shown). Hyaline degeneration is the most common type of degeneration of fibroids, which is characterized by the presence of homogeneous eosinophilic bands or plaques in the extracellular space, giving glassy appearance and losing the vortex structure, and can be used loosely to describe the histologic appearance of tissues.[37]

The images usually show uniform and spherical enlarged uterus, and internal inhomogeneous echo in the lesion, whereas for focal AM, the images usually show local inhomogeneous echo in the lesion compared with surrounding myometrium and with no certain boundary to the surrounding myometrium like a pseudocapsule circling around the fibroid; thus, these patients were difficult to be diagnosed with AM by TVUS. Although under TVESG examination, the myometrium of these patients showed broad areas of blue color and less green color, and the tissue stiffness values were significantly higher than those of normal myometrium, thus TVESG is superior in distinguishing AM from normal myometrium.

Higher Stiffness in AM Lesions as Compared to Fibroids and Normal Myometrium

We found that the stiffness of AM lesions is significantly higher than that of CM ($P = 1.5 \times 10^{-6}$) and of fibroid lesions ($P = .0006$; Figure 3A). The average tissue stiffness in CM, AM lesions, and UF tissues was 1.54 ± 0.82, 3.90 ± 1.00, and 2.68 ± 0.66, respectively. A multiple linear regression analysis incorporating age, menstrual phase, parity, preoperational use of hormonal therapy, source of tissues (AM or otherwise, fibroids or not), uterine size, and co-occurrence of endometriosis as covariates indicated that the tissue stiffness was only associated positively with the uterine size and AM and fibroid lesions ($P = .025$, $P = 3.5 \times 10^{-6}$, and $P = .049$, respectively; $R^2 = 0.58$) but not with other factors.

Within women with AM, there was no difference in lesional stiffness between focal and diffuse AM ($P = .82$;

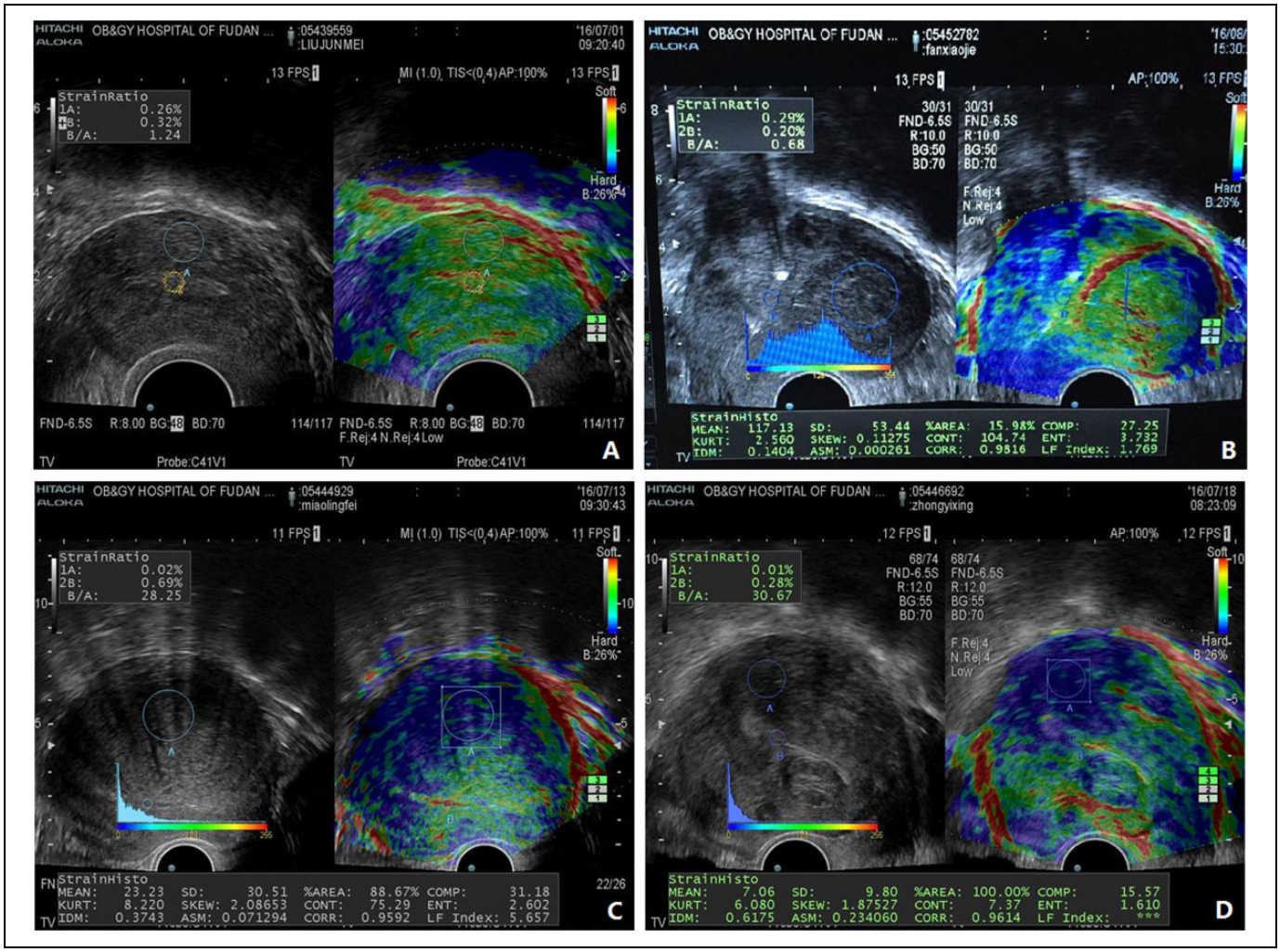

Figure 1. Representative transvaginal elastosonographic photos of different tissues: (A) normal myometrium shows the most green and less yellow color represents mild stiffness of the tissue; (B) uterine fibroid shows the most green and less blue color represents medium stiffness of the tissue, and there is also a typical red color pseudocapsule that circling around the fibroid; (C) diffuse adenomyotic tissue shows the broad area of blue color representing the even stiffer tissue; and (D) focal adenomyosis with co-occurrence of uterine fibroid. Focal adenomyotic tissue (the upper left area) shows the local area of blue color representing the even stiffer tissue. The uterine fibroid (the middle lower area) shows a typical red-colored pseudocapsule that circling around the fibroid. Typical serosal surface of uterus shown in red color could be seen in every uterus.

Figure 3B). Multiple linear regression analysis indicated that lesional stiffness was associated positively with the uterine size ($P = .024$; $R^2 = 0.14$) but not age, parity, menstrual phase, the type of AM, or the co-occurrence of endometriosis or of fibroids. Indeed, within women with AM, the lesional stiffness correlated positively with the uterine size ($r = 0.38$, $P = .024$; Figure 3C). The correlation coefficient was increased to 0.67 ($P = 3.9 \times 10^{-8}$; Figure 3D) if all participants were included.

Interestingly, for women with co-occurrence of AM and fibroids, the AM stiffness had no relationship with the fibroid stiffness ($r = -0.03$, $P = .92$). There was no difference in AM lesional stiffness between women with and without co-occurrence of UF ($P = .82$). There was no difference in lesional stiffness between women with moderate and heavy amount of menses ($P = .37$). The lesional stiffness appeared to correlate positively with the severity of dysmenorrhea, but the correlation did not reach statistical significance (Supplemental Figure 1A; $P = .11$ by the Jonckheere-Terpstra trend test).

Lesional Stiffness Correlates With the Extent of Fibrosis and Hormonal Receptor Expression in AM

We also evaluated the relationship, if any, between the lesional stiffness and the extent of fibrosis and the expression levels of E-cadherin (a marker of EMT), α-SMA (a marker for FMT), and of hormonal receptors ER-β and PR. We found that E-cadherin staining was seen in glandular epithelial cells of the control, eutopic, and ectopic endometrium and was localized in the cell membrane. α-Smooth muscle actin staining was seen primarily in the stroma of AM lesions. Estrogen

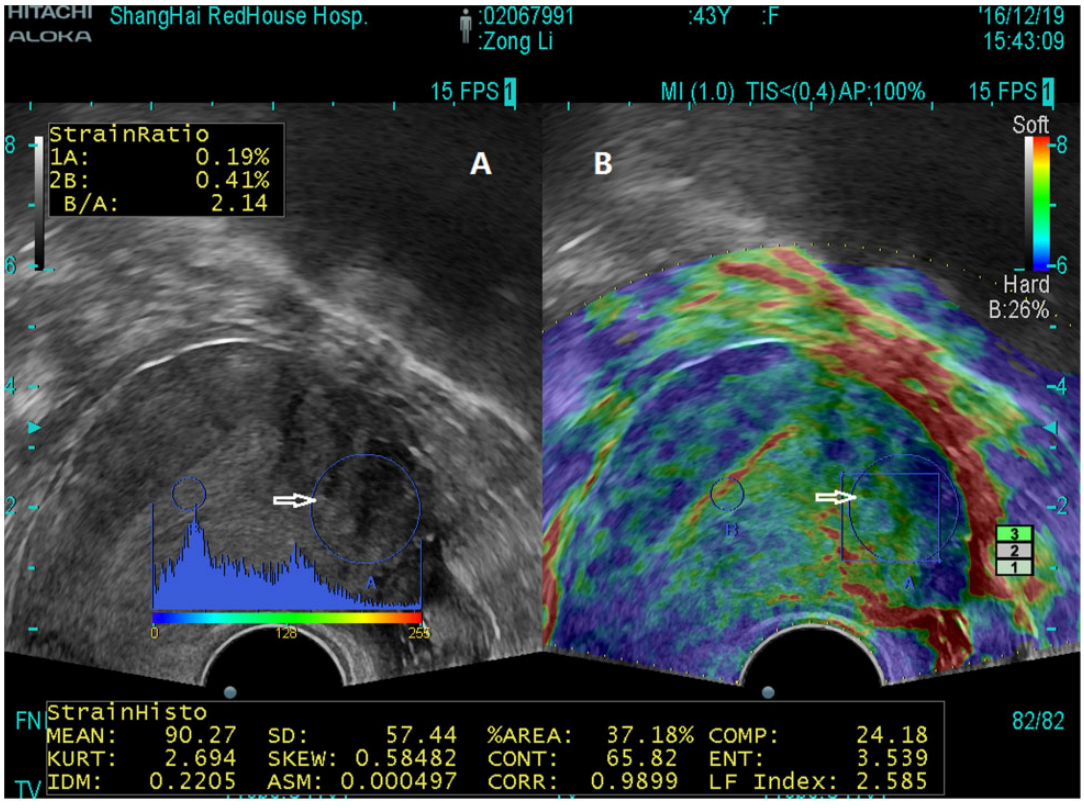

Figure 2. A. Transvaginal ultrasound image for a patient with a small uterus (59 × 58 × 57 mm) who complained of moderate dysmenorrhea with elevated CA125 level and was suspected with AM. The big circled area (white arrows) showed the ROI. The conventional B-mode TVUS image showed no sign that was consistent with a typical or spherical enlarged uterus or the presence of mild but not severe or obvious internal inhomogeneous echo in ROI. B, Transvaginal elastosonography image showing an increased stiffness value (LFI = 2.585) in the same ROI shown in the TVUS (white arrow), indicative of adenomyosis. AM indicates adenomyosis; LFI, liver function index; ROI, region of interest; TVUS, transvaginal ultrasound.

receptor β staining was seen mostly in glandular epithelial cells of the control, eutopic, and ectopic endometrium and was localized in the cytoplasm. Progesterone receptor staining was seen in the nuclei of both stromal and glandular epithelial cells of the control, eutopic, and ectopic endometrium (Figure 4).

We found that the lesional stiffness correlated closely with the extent of lesional fibrosis, as determined by the Masson Trichrome staining ($r = 0.90$, $P = 4.3 \times 10^{-13}$, with 1 apparent outlier removed, or $r = .92$, $P = 2.3 \times 10^{-15}$ without; Figure 5A). It also correlated negatively with the staining levels of E-cadherin and of PR ($r = -0.88$, $P = 4.7 \times 10^{-12}$, and $r = -0.88$, $P = 6.2 \times 10^{-12}$, respectively; Figure 5B and E) but positively with the staining levels of α-SMA and of ER-β ($r = 0.87$, $P = 2.8 \times 10^{-11}$, $r = 0.86$, $P = 5.9 \times 10^{-11}$, respectively; Figure 5C and D). Since the ratio of the extent of fibrosis versus the PR expression levels may indicate how responsive to hormonal treatment, we also plotted the lesional stiffness against the ratio and found that the 2 variables were highly correlated ($r = 0.93$, $P = 7.4 \times 10^{-16}$; Figure 5F). In all calculations, 1 patient, an apparent outlier, with the lowest lesional stiffness was removed. Further scrutiny of this patient revealed that she also had deep endometriosis and fibroids, severe dysmenorrhea, complained of heavy menses, and had mild anemia.

For all patients with AM and fibroids, the AM or fibroid lesional stiffness correlated closely with the extent of fibrosis ($r = 0.90$, $P < 2.2 \times 10^{-16}$; Figure 6A). Interestingly, the lesional stiffness in women with AM and endometrial stiffness in controls correlated negatively with the staining levels of E-cadherin and of PR ($r = -0.80$, $P = 5.4 \times 10^{-11}$, and $r = -0.82$, $P = 6.2 \times 10^{-12}$, respectively; Figure 5B and D) but positively with that of ER-β in *endometrium* ($r = 0.49$, $P = .0007$; Figure 6C). This may be due to the close correlation between lesional and endometrial immunostaining levels of E-cadherin ($r = 0.90$, $P = 3.2 \times 10^{-13}$), ER-β ($r = 0.91$, $P = 2.6 \times 10^{-14}$), and PR ($r = 0.91$, $P = 2.6 \times 10^{-14}$).

Similarly, the extent of lesional fibrosis and the E-cadherin staining also appeared to be correlate with the severity of dysmenorrhea, but the correlation did not reach statistical significance (Supplemental Figure 1B and C; $P = .28$ and $P = .26$, respectively, by the Jonckheere-Terpstra trend test). However, PR staining levels were found to correlate negatively with the severity of dysmenorrhea (Supplemental Figure 1D; $P = .029$ by the Jonckheere-Terpstra trend test), consistent with

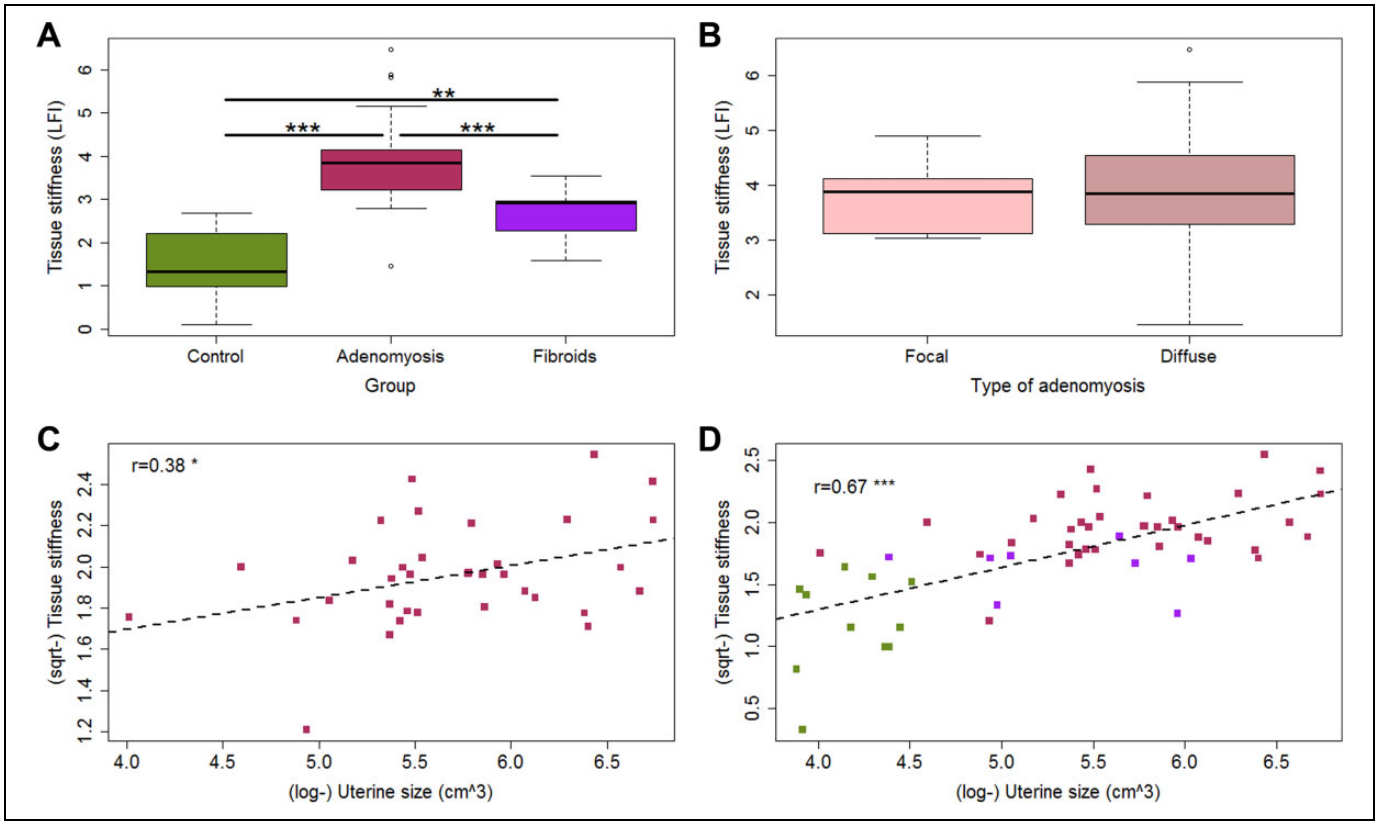

Figure 3. A, Box plots of tissue stiffness, as measured by TVESG, in different tissue groups. B, Box plots of lesional stiffness in different focal and diffuse adenomyosis. C, Scatter plot of lesional stiffness versus uterine size in patients with adenomyosis. The dashed line represents the regression line. The number shown is the correlation coefficient, along with the symbol showing the levels of statistical significance. D, Scatter plot of lesional stiffness versus uterine size in patients with adenomyosis (maroon-colored dots), uterine fibroids (purple-colored dots), and normal controls (olive drab-colored dots). The dashed line represents the regression line. The number shown is the correlation coefficient, along with the symbol showing the levels of statistical significance. *$P < .05$; **$P < .01$; ***$P < .001$. TVESG indicates transvaginal elastosonography.

previously reported.[38] Although individually the correlation between the VAS scores and lesional stiffness and extent of fibrosis and E-cadherin staining did not reach statistical significance, they were all consistent and, taken together, they suggest that patients with AM would experience more severe dysmenorrhea as lesions progress.

Tissue Stiffness in Fibroids and Its Relationship With the Extent of Fibrosis

No apparent relationship between the maximal fibroid size and the tissue stiffness was found ($r = -0.09$, $P = .69$; Figure 7A). However, the maximal fibroid size in patients with co-occurrence of AM was significantly smaller than that in patients without ($P = .0008$; Figure 7B). Fibroids in patients with co-occurrence of AM appeared to have lower tissue stiffness, but the difference was only marginally statistically significant ($P = .065$; Figure 7C). However, the tissue stiffness correlated positively with the extent of fibrosis in fibroids ($r = .85$, $P = 2.2 \times 10^{-7}$; Figure 7D), as in AM lesions. A multiple linear regression analysis incorporating age, menstrual phase, parity, preoperational use of hormonal therapy, co-occurrence with AM or not, uterine size, fibroid size, and the extent of fibrosis as covariates indicated that the tissue stiffness was only associated positively with the extent of fibrosis ($P = 2.2 \times 10^{-7}$, $R^2 = 0.73$; Figure 7D) but not the other factors.

Confirmation in the Larger Data Set

The characteristics of recruited patients in the 3 groups are listed in Table 2. As expected, the VAS scores correlated nearly perfectly with the VRS ($r = 0.99$, $P < 2.2 \times 10^{-16}$). Within women with AM, there was no difference in severity of dysmenorrhea between those with concurrent endometriosis ($P = .53$).

We found that the tissue stiffness of AM lesions is significantly higher than that of CM ($P < 2.2 \times 10^{-16}$) and of fibroid lesions ($P = 2.1 \times 10^{-6}$; Figure 8A), confirming our finding in the first part of this study. The average tissue stiffness in CM, AM lesions, and UF tissues was 1.42 ± 0.59, 3.66 ± 1.02, and 2.79 ± 0.69, respectively, and was remarkably similar to the first data set. A multiple linear regression analysis incorporating age, menstrual phase, parity, preoperational use of

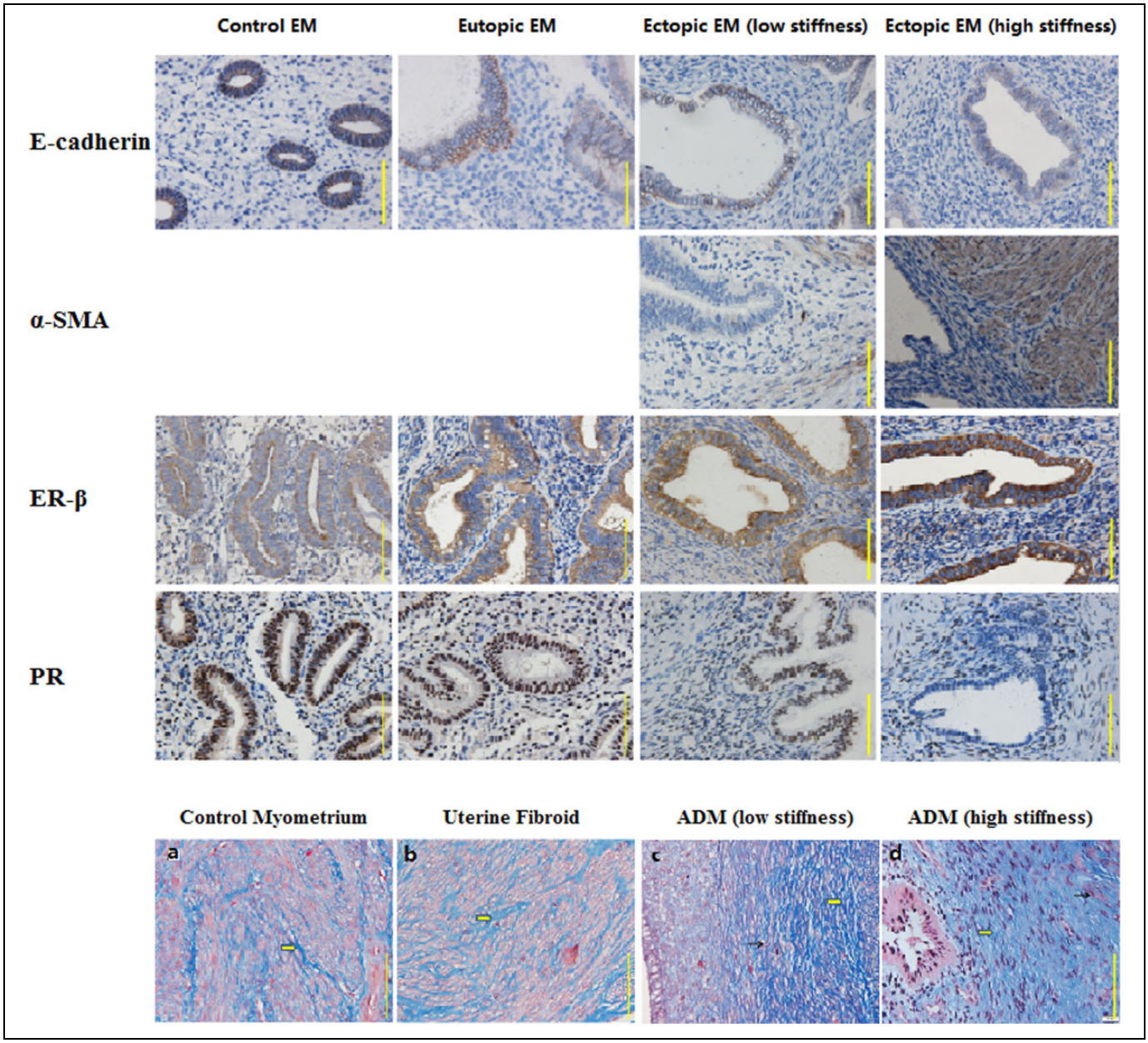

Figure 4. Upper panel: representative microphotographs on immunoreactivity against E-cadherin, α-SMA, ER-β, and PR in normal endometrium from controls, eutopic, and ectopic endometrium in patients with adenomyosis. Left to right column: control endometrium, eutopic endometrium, ectopic endometrium from a patient who has a lower lesional stiffness, and ectopic endometrium from a patient who has a higher lesional stiffness. Magnification in all figures: ×200. The scale bar represents 251 μm. Lower panel: representative Masson staining in different tissue groups as indicated: normal myometrium, uterine fibroids, ectopic endometrium from a patient who has a lower lesional stiffness, and ectopic EM from a patient who has a higher lesional stiffness. Magnification in all figures: ×400. The scale bar represents 125 μm. Yellow arrows point to collagen fibers stained in blue and while black arrows indicate muscle fibers stained in red. ADM indicates adenomyosis; EM, endometrium; ER-β, estrogen receptor β; PR, progesterone receptor; α-SMA, α-smooth muscle actin.

hormonal therapy, source of tissues (AM or otherwise, fibroids or not), co-occurrence of endometriosis or not, amount of menses, and the VAS on severity of dysmenorrhea as covariates indicated that the tissue stiffness was associated positively with AM lesions, fibroid lesions, amount of menses, and VAS ($P < 2.2 \times 10^{-16}$, $P < 2.2 \times 10^{-16}$, $P = .040$, and $P = 1.5 \times 10^{-4}$, respectively; $R^2 = 0.67$; Figure 7A).

Within women with AM, the lesional stiffness was correlated positively with the uterine size ($r = .38$, $P = 4.1 \times 10^{-5}$; Figure 8B). A multiple linear regression analysis incorporating age, menstrual phase, parity, preoperational use of hormonal therapy, co-occurrence of endometriosis or not, amount of menses, and the VAS on severity of dysmenorrhea as covariates indicated that the tissue stiffness was associated positively

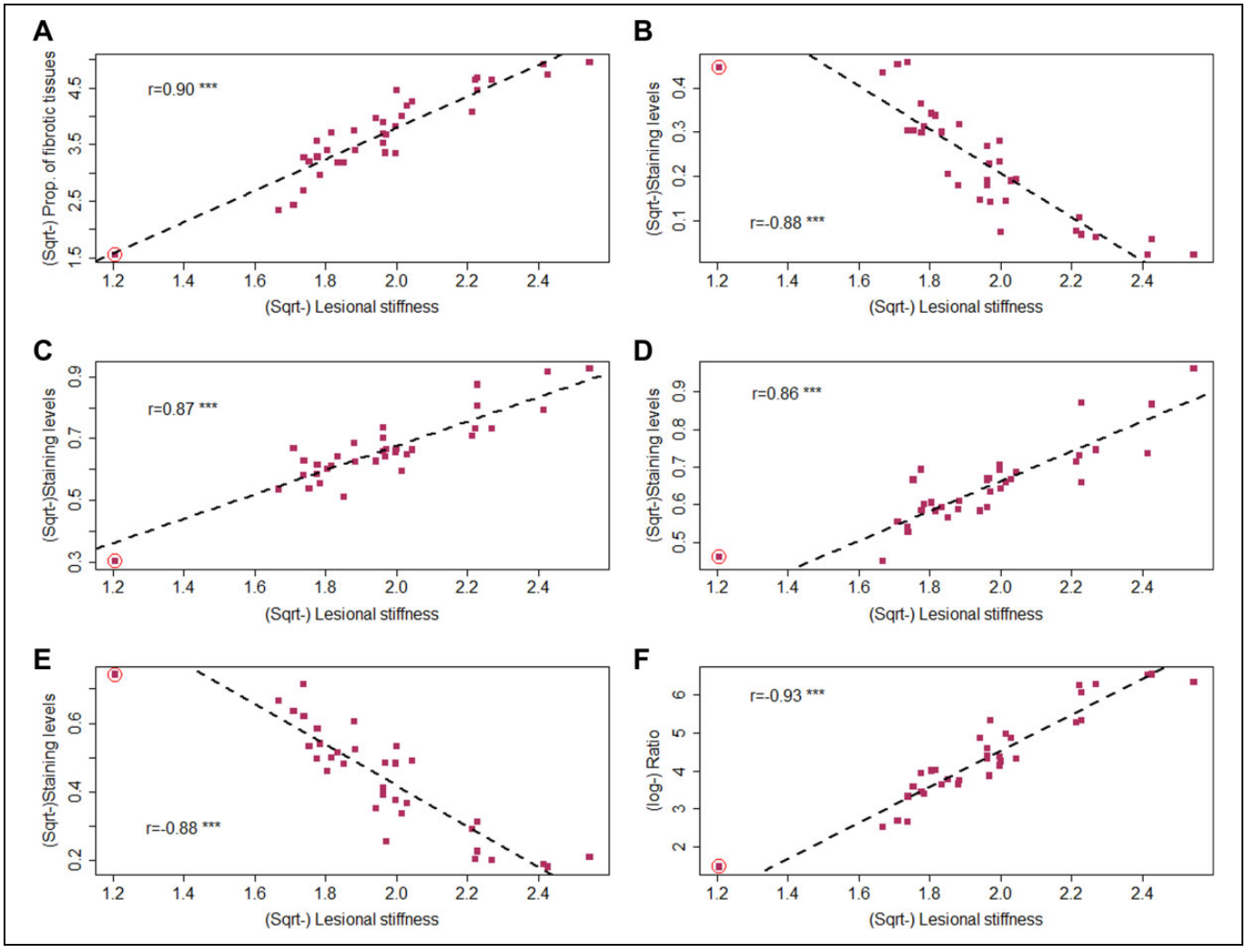

Figure 5. Scatter plot of lesional stiffness versus (A) the extent of lesional fibrosis, (B) E-cadherin staining levels, (C) α-SMA staining levels, (D) ER-β staining levels; (E) progesterone receptor staining levels, and (F) the extent of fibrosis versus PR expression ratio in patients with adenomyosis. The dashed line represents the regression line. The number shown is the correlation coefficient, along with the symbol showing the levels of statistical significance. *$P < .05$; **$P < .01$; ***$P < .001$. The spot with a red circle indicates that that data point was an outlier and was removed from calculation of correlation coefficient. ER-β indicates estrogen receptor β; PR, progesterone receptor; α-SMA, α-smooth muscle actin.

with the uterine size and VAS score ($P = 6.6 \times 10^{-5}$ and $P = 4.8 \times 10^{-5}$, respectively; $R^2 = 0.26$).

The lesional stiffness was higher in women who complained heavy menses than those who had light or moderate amount of menses ($P = .002$; Figure 8C). A multiple logistic regression analysis incorporating age, parity, preoperational use of hormonal therapy, uterine size, and lesional stiffness as covariates indicated that the amount of menses was associated positively with lesional stiffness ($P = .0036$; Akaike Information Criterion (AIC) = 140.4).

The lesional stiffness also correlated positively with the severity of dysmenorrhea (Spearman $r = .32$, $P = .0002$ for VRS and $r = 0.48$, $P = 8.8 \times 10^{-8}$ for VAS, respectively; the corresponding P values of Jonckheere-Terpstra tests were 3.1×10^{-4} and 9.5×10^{-9}, respectively; Figure 8D and E).

For women with AM and without, the tissue stiffness correlated positively with the uterine size ($r = .68$, $P < 2.2 \times 10^{-16}$; Figure 8F). There was no apparent relationship between tissue stiffness and the maximal fibroid size (Figure 9A). However, fibroids having hyaline degeneration had lower tissue stiffness ($P = .001$; Figure 9B). A multiple linear regression analysis incorporating age, parity, preoperational use of hormonal therapy, amount of menses, severity of dysmenorrhea, maximal fibroid size, and hyaline degeneration as covariates indicated that the fibroid stiffness was associated negatively with both age and hyaline degeneration ($P = .034$ and $P = 1.8 \times 10^{-6}$, respectively; $R^2 = 0.31$), even though marginally age did not seem to correlate with the lesional stiffness ($r = -0.15$, $P = .22$; Figure 9C).

For patients with UF, no apparent relationship was found between the lesional stiffness and the amount of menses ($P =$

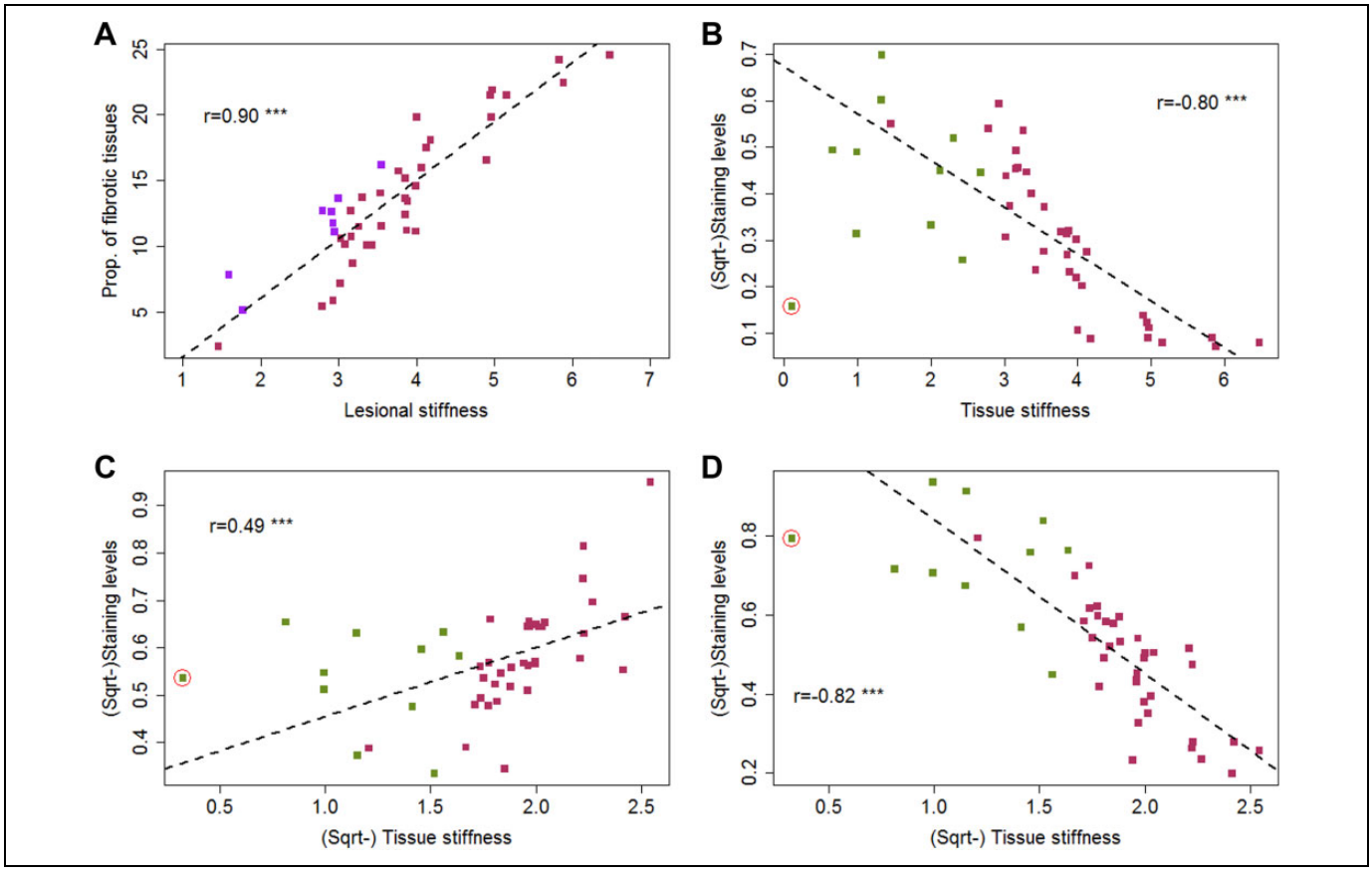

Figure 6. Scatter plot of lesional stiffness versus (A) the extent of lesional fibrosis in patients with adenomyosis (maroon-colored dots) and uterine fibroids (purple-colored dots), (B) E-cadherin staining levels, (C) ER-β staining levels, (D) progesterone receptor staining levels in patients with adenomyosis (maroon-colored dots) and control myometrium (olive drab-colored dots). The dashed line represents the regression line. The number shown is the correlation coefficient, along with the symbol showing the levels of statistical significance. *$P < .05$; **$P < .01$; ***$P < .001$. The spot with a red circle (B, C, and D) indicates that data point was an outlier and was removed from calculation of correlation coefficient. ER-β indicates estrogen receptor β.

.85), or the severity of dysmenorrhea ($P = .44$, by Jonckheere-Terpstra test), or between the maximal fibroid size and the amount of menses ($P = .83$), or the severity of dysmenorrhea ($P = .22$, by Jonckheere-Terpstra test). However, fibroids with hyaline degeneration were associated with heavy menses ($P = .015$). A multiple logistic regression analysis incorporating age, parity, preoperational use of hormonal therapy, maximal fibroid size, and hyaline degeneration as covariates indicated that the hyaline degradation was associated positively with heavy menses ($P = .013$; AIC = 66.1).

In CM, tissue stiffness appeared to increase with age, but the correlation coefficient was low and did not reach statistical significance ($r = 0.11$, $P = .23$; Figure 9D).

Discussion

In the last decade, growing numbers of ultrasonic diagnostic instrument manufacturers have added ESG to their ultrasound systems, and today, the majority of them offer some sort of elastography or tissue stiffness imaging on their products.[39] Given the need for accurate diagnosis of AM and of differentiate diagnosis of AM and UF, and given in particular our recent understanding that AM lesions are wounds undergoing ReTIAR that progress progressively to fibrosis through EMT, FMT, and SMM,[11-14] TVESG is well suited for diagnosing AM, especially for differential diagnosis.

Our study demonstrates that TVESG is superior to conventional TVUS for these purposes. Moreover, the correlation between lesional stiffness and lesional expression levels of hormonal receptors such as ER-β and PR strongly suggests the prospect of using the TVESG findings to guide the choice of the best treatment modality for the patient since the action of progesterone is mediated through PR. Apparently, higher lesional stiffness would have lower PR expression, which means that the patient would be less likely to respond to progestin treatment. The close correlation between lesional stiffness and expression levels of markers of EMT, FMT, and the extent of fibrosis in lesions underscores the fact that the ReTIAR theory captures the essence of the natural history of AM lesions, and TVESG images simply provide an estimate as how advanced the lesion has progressed.

Our data consistently show that the stiffness of AM lesions is significantly higher than that of fibroid tissues, which in turn

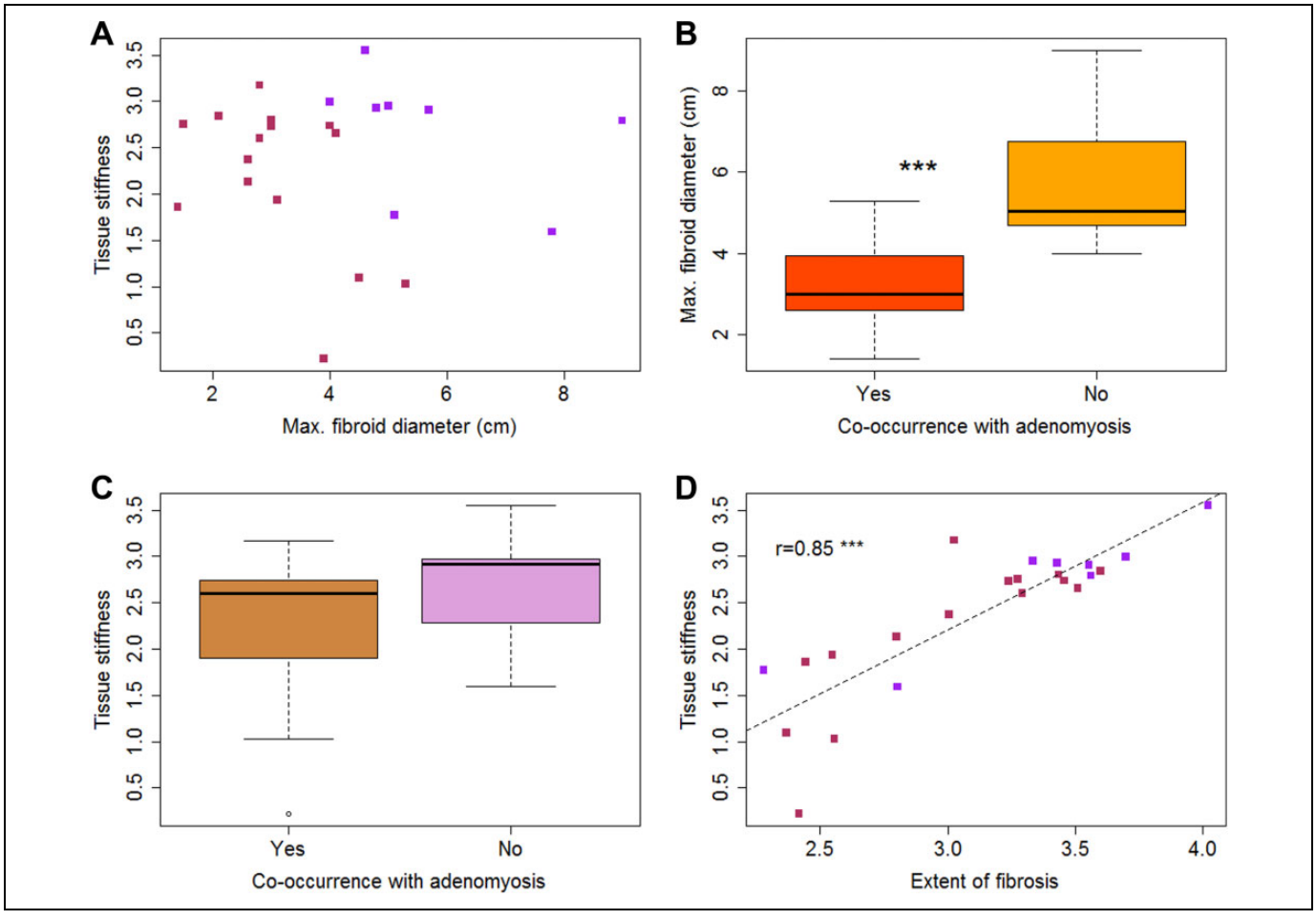

Figure 7. A, Scatter plot of tissue stiffness of the uterine fibroids versus the maximal size of the fibroid in patients with uterine fibroids. The purple-colored dots represent data from patients with uterine fibroids only, whereas the maroon-colored dots represent data points from patients with uterine fibroids and coexisting adenomyosis. B, Box plot of the maximal fibroid size among patients with and without coexisting adenomyosis. C, Box plot of tissue stiffness in uterine fibroids among patients with and without coexisting adenomyosis. D, Scatter plot of tissue stiffness of the uterine fibroids versus the extent of fibrosis in patients with uterine fibroids. The purple-colored dots represent data from patients with uterine fibroids only, whereas the maroon-colored dots represent data points from patients with uterine fibroids and coexisting adenomyosis. The dashed line represents the regression line. The number shown is the correlation coefficient, along with the symbol showing the levels of statistical significance. ***$P < .001$.

is significantly higher than that of normal myometrium. Since the content and composition of the ECM determine the tissue stiffness, and since our data are consistent with the well-documented higher collagen I, III, and V in fibroids as compared with that of normal myometrium,[40-44] especially collagen I,[42,43] which is the toughest collagen among all types of collagens. Using the data shown by Iwahashi and Muragaki,[41] the average ratio of intensity of type I collagen in the quantification of protein in fibroids was 0.59 versus 0.48 in normal myometrium, and for type V collagen, 0.13 versus 0.06, yielding a ratio of 1.23 and 2.17 for type I and type V collagens. Based on our data presented, the ratio of the average tissue stiffness in fibroids versus normal myometrium was 1.74 for the IHC data set and 1.96 for the validation data set, roughly in agreement with the protein data reported in the study by Iwahashi and Muragaki.[41]

The higher stiffness in AM lesions than in fibroids may stem from the fact that, unlike fibroids, AM lesions undergo ReTAIR, resulting in progressive EMT, FMT, SMM, and fibrosis.[13,14] Consequently, AM lesions may have more abundant collagen I fibers, rendering lesions stiffer than fibroids.

The positive correlation between lesional stiffness and uterine size in women with AM is consistent with the data corroborating the ReTAIR theory, since AM stromal cells are progressively transdifferentiated into smooth muscle cells due to the TGF-β1,[11,13,14] resulting in increased smooth muscle cell population and thus uterine size.

We note that there are still ample rooms for improvement for diagnosing AM using TVESG. First, the stiffness measure, that is, LFI, we used in this study is a built-in stiffness measurement implemented in the Hitachi machines that is displayed automatically after the ROI is positioned. Even

Table 2. Characteristics of Recruited Patients With Adenomyosis or Uterine Fibroids and Without (Controls) for the Second Part of This Study.

Item	Control (n = 130)	Adenomyosis (n = 112)	Uterine Fibroids (n = 67)	Statistical Significance[a]
Age, years	Mean (SD) = 39.4 (6.5) Median (range) = 39.5 (25-50)	Mean (SD) = 41.4 (6.1) Median (range) = 42.0 (29-52)	Mean (SD) = 41.2 (6.8) Median (range) = 43.0 (25-52)	.027
Menstrual phase				
Proliferative	74 (56.9%)	75 (67.0%)	40 (59.7%)	.26
Secretory	56 (43.1%)	37 (33.0%)	27 (40.3%)	
Parity				
0	21 (16.2%)	16 (14.3%)	10 (14.9%)	.53
1	82 (63.1%)	73 (65.2%)	49 (73.1%)	
≥2	27 (20.7%)	24 (21.4%)	8 (11.9%)	
Severity of dysmenorrhea				
None	121 (93.1%)	12 (10.7%)	53 (79.1%)	$<2.2 \times 10^{-16}$
Mild	8 (6.2%)	17 (15.2%)	11 (16.4%)	
Moderate	1 (0.8%)	28 (25.0%)	3 (4.5%)	
Severe	0 (0.0%)	55 (49.1%)	0 (0.0%)	
Visual Analog Scale scores on dysmenorrhea	Mean (SD) = 0.12 (0.53) Median (range) = 0 (0-4)	Mean (SD) = 5.49 (2.75) Median (range) = 6 (0-9)	Mean (SD) = 0.66 (1.38) Median (range) = 0 (0-5)	$<2.2 \times 10^{-16}$
Uterus size, cm^3	Mean (SD) = 54.2 (17.9) Median (range) = 51.0 (21.1-93.1)	Mean (SD) = 186.2 (132.3) Median (range) = 141.5 (51.7-761.0)	NA	NA

Abbreviations: NA, not applicable; SD, standard deviation
[a]*P < .05; **P < .01; ***P < .001; NS: P > .05. Wilcoxon rank test was used for age while for other data Fisher exact test was used.

though LFI correlated nicely with the extent of lesional fibrosis, it was designed to measure liver fibrosis, not the extent of fibrosis in AM per se. Hence, it may not be perfectly optimal for measuring uterine stiffness. Even for quantification of liver fibrosis, the strain ESG technology is still evolving rapidly.[45] Second, this study used strain ESG, in which the tissue stiffness measurement reading may depend on the amount of pressure that the operator makes on the transducer. Other ESG method, such as the shear wave ESG, that induces and monitors shear wave propagation in tissues and reports a quantitative value directly related to the stiffness might be less operator dependent.

TVESG is ideally suitable for noninvasive diagnosis of AM and also for differential diagnosis. Aside from its ability to spot AM lesions in small uteri as we experienced, its stiffness measurement is unaffected by the menstrual phase. The lesional stiffness correlated highly with the extent of lesional fibrosis and the severity of symptoms. In addition, the lesional stiffness correlates positively with ER-β expression levels but negatively with the PR expression levels. The latter finding indicates that, with increased lesional stiffness indicative of higher fibrotic content, the lesions are not likely to be responsive to progesterone due to increased ER-β expression and reduced PR expression.[46] Since increased extent of lesional fibrosis is also associated with decreased vascularity and more progressive epigenetic changes,[47] these patients are unlikely to be responsive to hormonal treatment. In other words, TVESG not only provides a more accurate diagnosis of AM than conventional TVUS but also provides additional information that can be used to decide the best treatment modality for the patient. Of course, future studies are needed to see whether this inference is true or not.

Our finding that AM lesions are about 2.6-fold higher stiffness than that of healthy myometrium is consistent with the report using shear wave elastography, which reports that the mean stiffness in AM and control groups was 72.7 kPa and 24.4 kPa, respectively, or about 3.0-fold difference.[27]

Our experience with the TVESG indicates that it is particularly suitable for diagnose patients with AM co-occurring with or without UF, or those with AM but with a smaller uterus. The colored display also is very intuitive, often giving the operator an instant spotting of a suspected AM lesion. Since lesional stiffness essentially captures the developmental stage of the lesion, we know immediately as how advanced the lesional developmental stage is. By the same ReTAIR theory, TVESG can be also used for diagnosing deep endometriosis since the 2 conditions share the hallmark of cyclic bleeding and the same developmental course[11,36,47,13] as has been shown to be feasible.[48]

Our results are somewhat different from those by Frank et al[26] who report that the "lesion index" in fibroids are twice as high as that of healthy uterine tissues but the index in AM is about half as that of as healthy tissues. Different machines aside, there are several notable differences. First, their index was defined as the maximum ratio of ROI3 versus ROI4 (ie, lesion index = max (ROI3/ROI4)), where ROI3 was placed in healthy homogeneous tissue and ROI4 was placed on an equal level in a myoma or an area of AM (but left undefined for normal uteri). By careful parsing the definition of their "lesion index," it is apparent that the index is roughly the inverse of the

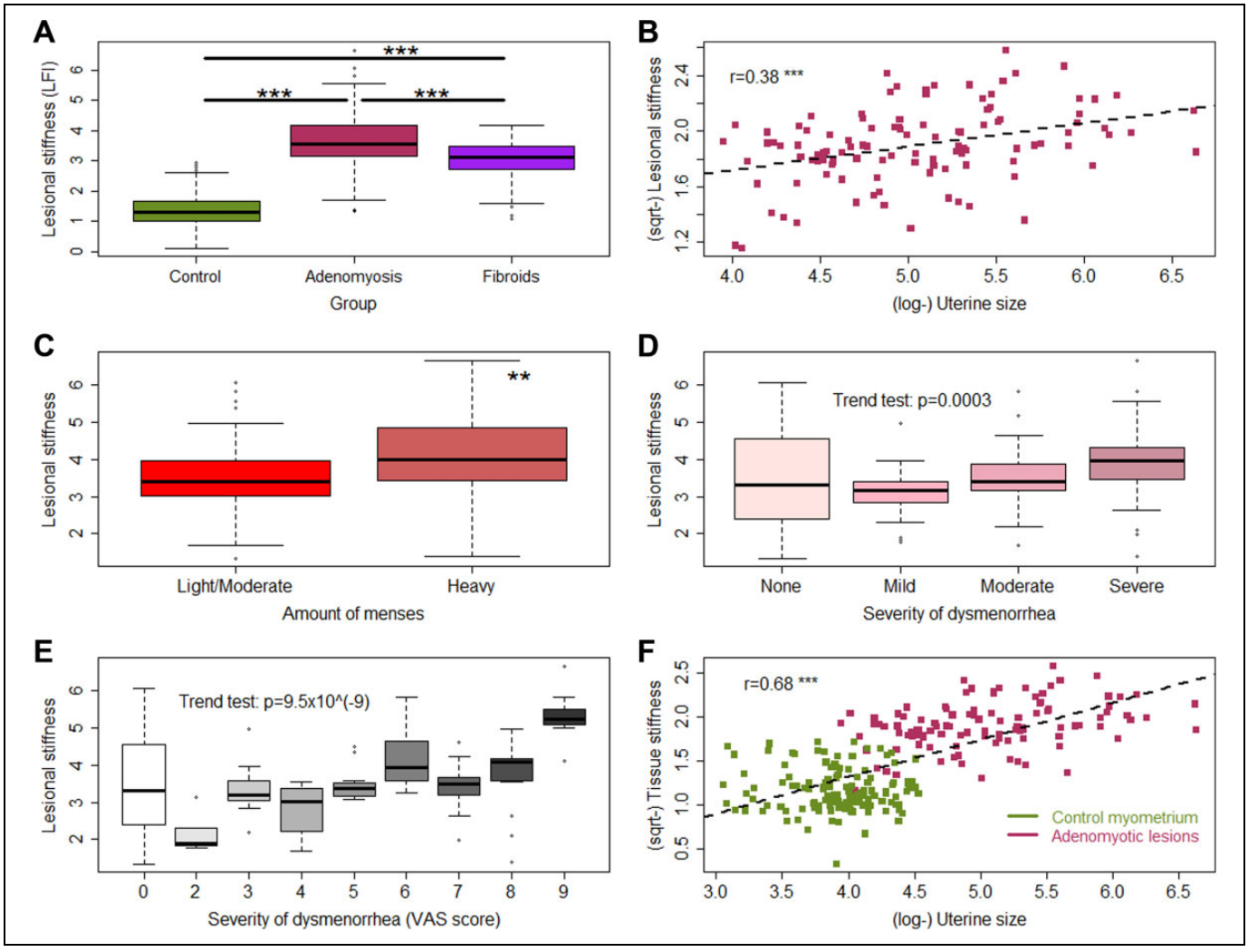

Figure 8. A, Box plot of tissue stiffness, as measured by TVESG, in different tissue groups for the confirmation data set. B, Scatter plot of lesional stiffness versus uterine size in patients with adenomyosis. The dashed line represents the regression line. The number shown is the correlation coefficient, along with the symbol showing the levels of statistical significance. C, Box plot of lesional stiffness in women with adenomyosis who reported light/moderate amount of menses and heavy menses. D, Box plot of lesional stiffness in women with adenomyosis who reported different severity of dysmenorrhea on the Verbal Descriptor Scale. E, Box plot of lesional stiffness in women with adenomyosis who reported different severity of dysmenorrhea on the Visual Analog Scale. F, Scatter plot of lesional stiffness versus uterine size in patients with adenomyosis (maroon-colored dots) and uterine fibroids (purple-colored dots). The dashed line represents the regression line. The number shown is the correlation coefficient, along with the symbol showing the levels of statistical significance. *$P < .05$; **$P < .01$; ***$P < .001$. TVESG indicates transvaginal elastosonography.

lesional stiffness of either fibroids or AM relative to their adjacent myometrial stiffness. With this interpretation, their result is that the lesional stiffness in AM (relative to their adjacent myometrium) is the highest, followed by the control uteri, and is the lowest in fibroids. With this interpretation, the relative AM stiffness is 2.7-fold higher than control uteri, which is remarkably similar to our number. However, their finding that the relative fibroids stiffness is merely 0.45 of that control uteri is surprising and apparently is at odds with the well-known fact that fibroids are related to excessive production and deposition of stiff, disorganized, and exceptionally stable ECM, which should be stiffer than normal myometrium.[49] Second, they used ratios of stiffness in 2 ROIs in the uteri to define stiffness, in contrast to the stiffness on 1 ROI that we used in this study, which was evaluated as a single measurement and has been well tested in the setting of liver fibrosis. Intrinsically, the ratio of 2 random variables may be more difficult to quantify than a single one. In addition, their use of the maximum means that the minimum lesional stiffness relative to myometrium is presented, and the minimum lesional stiffness may not useful for the choice of the best treatment modality since it is conceivably the maximum lesional stiffness determines the most advanced lesional development stage, which may determine the responsiveness to medication.

Our study has some limitations. First, the sonographer was not blinded, and there might have been some selection bias

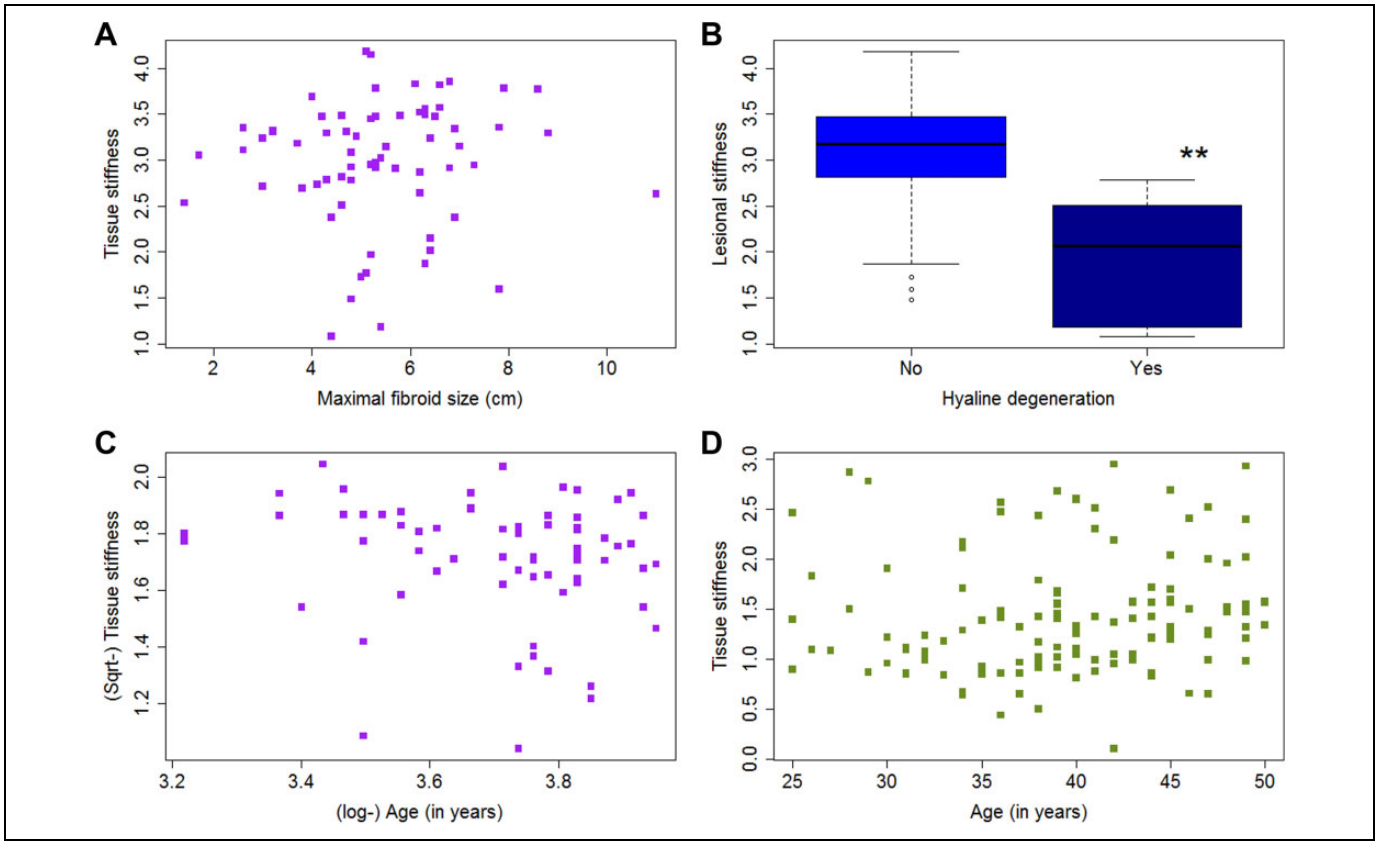

Figure 9. A, Scatter plot of tissue stiffness of the uterine fibroids versus the maximal size of the fibroid in patients with uterine fibroids. B, Box plot of tissue stiffness between fibroids with and without hyaline degeneration in women with uterine fibroids. C, Scatter plot of tissue stiffness of the uterine fibroids versus age in patients with uterine fibroids. D, Scatter plot of tissue stiffness in myometrium versus the age of the control subjects who were healthy.

due to the choice of patients who already underwent TVUS evaluation. However, with 22 years of gynecologic sonography experience under her belt, these biases are unlikely to affect her diagnosis or impact on our conclusions. Second, the lack of histologic confirmation in the second part of our study may contain some misclassification. However, given the report that the accuracy of TVUS-based diagnosis of AM is on par with that of MRI,[50] which is widely accepted for diagnosis, the majority of diagnosed cases had to be correct, especially in the hands of a veteran sonographer. Again, some misclassification, if exists, is not likely to change our conclusions.

In summary, we found TVESG is superior to conventional TVUS in diagnosing AM and in differential diagnosis of AM and UF. In addition, lesional stiffness as measured by TVESG correlates closely with the extent of lesional fibrosis, and with expression levels of markers of EMT and FMT, and also of hormonal receptors. The lesional stiffness also correlates with the severity of symptoms in patients with AM . These data demonstrate that TVESG not only can diagnose AM more accurately than conventional TVUS and instantly provides an assessment of the developmental stage of the lesions but also may be used to guide the choice of the best treatment modality for the patient. Future studies are warranted to search for an optimal algorithm and provide an estimate of the sensitivity and specificity of TVESG.

Authors' Note

Xishi Liu, Ding Ding, and Yunyun Ren contributed equally to this work. SWG conceived and designed the study, performed data analysis and data interpretation, and drafted the manuscript. Ding Ding carried out all IHC and histologic analyses, Xishi Liu provided technical and clinical guidance to and also oversaw the project, and Yunyun Ren carried out all imaging analyses and diagnostics. All participated in writing and approved the final version of the manuscript.

Acknowledgments

The authors thank Dr Fang Wu from the Shanghai Branch of the Hitachi Company for loaning the ultrasound machine to us and her expert assistance.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work

was supported in part by grants 81471434 (SWG), 81530040 (SWG), 81771553 (SWG), and 81370695 (XSL) from the National Science Foundation of China.

Supplementary Material

Supplementary material for this article is available online.

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